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CHARACTER COMBINATIONS AND DISTRIBUTION IN THE GENUS *FRITILLARIA* AND ALLIED GENERA

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INTRODUCTION

The genus *Fritillaria* is placed with six other genera in the tribe *Tulipeae* which Krause, following Engler, raised (with slight modifications of the generic content) to the rank of a subfamily under the name *Lilioideae* (Krause in Pflanzenfam. **15 A.** 1930, ed. 2). Bentham and Hooker, but not Krause, include *Gagea* in this group. In this paper we are chiefly concerned with the genus *Fritillaria* but reference will be made to species of *Korolkowia, Lilium, Nomocharis,* and *Notholirion.* The other genera, associated by the above authors with *Fritillaria,* namely, *Tulipa, Erythronium, Lloydia,* and *Calochortus,* and also *Gagea* (associated with *Allium* in Krause's classification) are more distinct and are not considered further here. The genera *Korolkowia, Nomocharis,* and *Notholirion* are not recognized as such by Bentham and Hooker, but *Nomocharis* is accepted by Krause.

Fritillaria consists of about 100 known species and though there may still be some reductions there are probably some new ones still to be discovered and described. It is widely spread throughout Europe, N.W. Africa, temperate Asia, and western North America. A number of fairly well-marked sections can usefully be recognized. The largest is *Eufritillaria* typified by *F. meleagris,* the common snakeshead, the standard species of the genus and the one it is fair and convenient to take as "typical" without supposing any special phylogenetic scheme. The section *Petilium* includes the well-known *F. imperialis,* the crown imperial. *Theresia,* with *F. persica, Rhinopetalum* with *F. karelinii,* and *Liliorhiza* with *F. lanceolata* are other subdivisions to species of which references will be made.

THE CONCEPT OF RETICULATION

It is proposed to use various species of *Fritillaria* to illustrate the phenomenon known as "reticulation." The term itself, as used in the specialized sense about to be described, is not entirely a happy one since a network usually shows a regular pattern. In the present context it is the occurrence in different combinations of characters described in the same or similar terms that is intended by "reticulation." The segregation and recombination of characters (and of their correlated genes) is, of course, a basic principle of Mendelian inheritance. Moreover, Gates's suggestions regarding "parallel mutations" and Vavilov's concept of

"homologous variation" have a direct bearing on some of the problems connected with reticulations as they present themselves to the taxonomist. These problems, as a whole, are, however, more complicated than has usually been recognized and, moreover, may be of fundamental importance in evolutionary theory.

The concept of "reticulation" is not a new one since it dates from long before the general acceptance of evolutionary theories. The image of a net was employed by Donati (1750) to represent the ordered complexity of nature. Linnaeus (1756) notes that plants show affinities on all sides, like a district on a geographical map. Robert Brown (1810) refers to organisms being "reticulatim" rather than "catenatim" connected. Cuvier (1828) uses the French word "réseau" as a symbol of the organic world. Further general references to and remarks concerning reticulation in plants will be found in Turrill: Taxonomy and Phylogeny, in Bot. Rev. **8**: 1942, especially pp. 259 seq.

Characters of Fritillaria Used in the Study of Reticulation

The following characters in *Fritillaria* have been selected for this present study of reticulation. They do not exhaust the characters that could be used but include some from all parts of the plants and many of those considered of importance by taxonomists. Not all but a reasonable selection of species of the genus are named in this account.

Bulbs. Two fleshy scales of the current season are present in *F. meleagris, F. verticillata,* and the majority of species of the genus. Several fleshy scales are present in *F. imperialis, F. atropurpurea, F. liliacea, F. agrestis,* and *F. dagana.* "Rice-bulbils" occur in *F. lanceolata, F. mutica, F. multiflora, F. pudica* and other Northwest American species, *F. camschatcensis* from E. Asia and N.W. America, and *F. davidii* from Sikang, W. China.

Leaves. At least the upper leaves are spirally arranged on the flowering stems in *F. meleagris, F. tubiformis, F. persica, F. libanotica, F. pluriflora, F. pudica,* etc. The leaves are typically opposite in *F. involucrata* and *F. pontica.* Whorled leaves are characteristic of *F. imperialis, F. camschatcensis* (often), *F. verticillata, F. maximowiczii, F. recurva, F. biflora, F. lanceolata,* and *F. mutica.* Most species have non-cirrhose leaves, as in *F. meleagris, F. pyrenaica,* and *F. pontica.* Cirrhose leaves characterize *F. ruthenica, F. verticillata, F. olgae* (upper leaves), *F. usuriensis, F. walujewi,* and *F. cirrhosa.* Unusually narrow leaves occur in *F. lusitanica* var. *stenophylla, F. fleischeri, F. forbesii,* and *F. meleagroides.*

Tepals. Tessellations are present in *F. meleagris, F. macedonica, F. tubiformis, F. latifolia, F. cilicico-taurica, F. przewalskii,* and some other species. They are absent in such species as *F. pudica, F. obliqua, F. glaucoviridis, F. libanotica,* and many other species.

Well marked fasciae (bands differently coloured from the rest of the tepals) occur in *F. lusitanica, F. graeca, F. messanensis, F. oranensis,* and other species but are absent from the majority as they are from *F. meleagris.*

Spreading to reflexed tips occur in *F. acmopetala, F. pyrenaica,* and *F. reflexa.*

Pale yellow or more or less whitish yellow tepals characterize the species or certain variants within the species: *F. meleagris, F. tubiformis, F. latifolia, F. lutea, F. sibthorpiana, F. pudica.*

Nectaries. Long more or less narrow furrows are found in *F. lanceolata, F. pluriflora, F. camschatcensis, F. drenovskii,* and *F. olivieri.* Elongated pits occur in *F. meleagris, F. acmopetala, F. davisii, F. tuntasia, F. fusca, F. graeca, F. oranensis,* and *F. caucasica.* Pits broader in relation to their length are found in *F. ehrhartii, F. glaucoviridis, F. messanensis, F. pontica, F. pudica,* etc. Saucer-like pits characterize *F. imperialis* and *F. raddeana.* Elongated subspurred nectaries are found in *F. bucharica* and *F. pallidiflora* while there are more definite furrows and spurs in *F. karelinii.*

Androecium. Many of the species of

Fritillaria have papillose or hairy filaments but these are glabrous in *F. dagana, F. verticillata, F. camschatcensis* and all Western American species. Of particular interest are *F. armena* with papillose and *F. caucasica* with glabrous filaments, since these two taxa are very closely related and may be best considered as variants of one species. The anthers in most fritillaries are basifixed or very slightly dorsifixed when quite mature. In *F. bornmulleri, F. pudica, F. camschatcensis,* and some other species they are semi-versatile (at least sometimes) and in *Kolreuteria* they are versatile almost as they are in *Lilium.*

Gynoecium. Definitely trifid styles are found in most species of *Fritillaria,* as in *F. meleagris, F. lanceolata,* etc. Shortly three-lobed styles occur in *F. pluriflora, F. striata, F. imperialis,* and *F. aurea.* Entire styles distinguish *F. tuntasia, F. bucharica, F. ehrhartii, F. libanotica, F. citrina, F. dasyphylla, F. glaucoviridis* (style thick), *F. pudica, F. striata,* and some other species.

Capsules. Winged capsules occur in *F. recurva, F. pallidiflora, F. mutica, F. multiflora, F. libanotica, F. lanceolata, F. imperialis, F. glaucoviridis, F. dagana,* etc. Most species of which ripe capsules have been studied have no capsular wings. Such include: *F. meleagris, F. sibthorpiana, F. tuntasia, F. ruthenica, F. pyrenaica, F. purdyi, F. pluriflora, F. graeca, F. liliacea, F. agrestis, F. acmopetala,* and others. It is interesting to note that *F. pontica* has, and the otherwise very similar *F. involucrata* has not, wings to the capsules (judging from available specimens). Short "shoulder horns" occur in *F. pinetorum, F. arianum, F. karelinii, F. libanotica,* and *F. glaucoviridis.*

Discussion

The taxonomic value of bulb characters has been previously stressed by the writer. Here it is only necessary to draw attention to the geographical distribution of species with multiple scales and "rice-bulbils." All known American species have either more than two bulb scales and/or "rice-bulbils." However, *F. camschatcensis* with "rice-bulbils" extends from western America into eastern Asia, while *F. dagana* and *F. imperialis* with several fleshy scales and *F. davidii* with "rice-bulbils" are Asiatic species.

The above analysis of characters can be extended very considerably but enough facts have been given to prove that many different character combinations occur and yet there are apparently certain limitations to completely free association and some marked correlations or "linkages" that are significant. A table of certain character combinations for ten species (5 Old World, 1 Old and New World, 4 New World) will emphasize some of the points already made and will provide illustrations for the discussion that follows. This discussion is frankly speculative and any conclusions reached are tentative. Our knowledge of the genetics and autecology of species of *Fritillaria* is very meagre indeed and until detailed experimental and field studies result in sufficient facts regarding inheritance and selection one can only apply analogy (using data from other genera) or reasonable possibilities. For the solution of some of the general problems of which examples are provided by the present study, *Fritillaria* will probably not provide the most suitable material. Nevertheless, to attempt explanations is one of the best ways of focussing attention on the problems themselves.

"Reticulation," as here understood, is based on taxonomic characters and it is essential to distinguish true reticulation from false reticulation and in the former to realize that genetically two kinds occur. We then have the following scheme:

1. *True reticulation.*
   - A. With the same kinds of genes present.
   - B. With different genoms giving the same characters.

2. *False reticulation.* With characters described in similar terms but either

not strictly homologous morphologically or attained by very different phylogenetic routes.

It is also desirable to consider the limitations of free association of characters and these may be due to:

i. Inheritance of a common genotype with absence of mutations in the loci concerned.

ii. Selection by the external environment against certain character combinations and for certain other combinations.

iii. Regulations by the laws of logic, chemistry, or physics which make certain characters or character combinations impossible.

There is no general and obvious regular pattern in the reticulation of characters found in *Fritillaria* and related genera. This makes one suspect that any explanation must involve a number of more or less independent "causes." There are, however, a number of restricted or local regularities in which there is some degree of correlation constancy and clues to the "causes" of reticulation may be found by a study of these. Bearing in mind the above tentative scheme it is worth while to see how it has been worked out for *Fritillaria* and allied genera.

The first obvious correlations are those in the American group of species. All of them have more than two current season store scales to the bulb with or without "rice-bulbils," non-cirrhose leaves, and glabrous filaments. In addition whorled leaves occur in a higher proportion of species than in the European-Asiatic fritillaries. It is interesting to note that whorled leaves are characteristic of many American species of *Lilium*. None of these characters is confined to American species and in addition there are some striking parallelisms with Old World species. Thus, the American *F. pudica* has entire styles like the European-Asiatic *Olostyleae* of Boissier (Fl. Or. **5**, 185 seq.: 1884) and, indeed, is remarkably similar, except in bulb characters, to *F. sibthorpiana*. *F. pinetorum* (*F. atropurpurea* var. *pinetorum*) has capsules with shoulder horns like those in *F. glaucoviridis*. It is important to note that *F. camschatcensis*, which in various characters approximates to species of *Lilium* (see Bot. Mag.

TABLE 1. *Character combinations for ten species of* Fritillaria

	2 bulb scales	Several bulb scales and/or "rice-bulbils"	Leaves spiral	Leaves opposite	Leaves whorled	Leaves cirrhose	Leaves not cirrhose	Tepals tesselated	Tepals not tesselated	Nectaries pits	Nectaries furrows	Filaments papillose	Filaments glabrous	Styles trifid	Styles entire	Capsules winged	Capsules not winged	Capsules horned	Capsules not horned	Distribution	Chromosome number (2n)
F. meleagris	X		X				X	X		X		X		X			X		X	E	24
F. pontica	X			X			X		X	X		X		X		X			X	EA	24
F. verticillata	X				X	X		X		X			X	X		X		X		A	—
F. glaucoviridis	X		X				X		X	X		X			X	X		X		A	—
F. pallidiflora		X?	X				X		X	X			X	X		X			X	A	24
F. imperialis		X			X		X		X	X			X	X		X			X	A	24
F. camschatcensis		X			X		X		X		X		X	X			X		X	AAm	24 36
F. recurva		X			X		X		X		X		X	X		X			X	Am	24
F. purdyi		X	X				X	X		X			X	X			X		X	Am	24
F. pudica		X	X				X		X	X			X		X		X		X	Am	24 26 39

In the "distribution" column E = Europe, A = Asia, Am = America.

N.S. t. 63: 1949), agrees in the correlated characters with the American and not with the Asiatic species of *Fritillaria* and is the species linking the geographical ranges of American with Asiatic fritillaries. The resemblances within the American group of species are quite probably due to inheritance of a common genetic basis but the reticulation of characters with European species is probably of diverse and independent origin. Thus the entire style character is possibly the result of "parallel mutations" but whether of the same or of different genes (1 A or 1 B in our scheme) is quite uncertain. Glabrous filaments in all the American species give a reticulation with glabrous filaments in some Old World species. There can be no doubt that the filament difference between *F. armena* (papillose) and *F. caucasica* (glabrous) is very simply genetic but "loss" of a character is not always due to the same gene change and if the glabrous state of the filaments in the American species be correlated with different gene changes from those in the Old World species we have an example of 1 B in our scheme. All American species of *Lilium* examined have glabrous filaments.

Another interesting group of three species (or three subspecies or varieties of one species) is composed of *F. involucrata, F. ionica,* and *F. pontica.* These have most characters in common and in particular have the cauline leaves generally opposite and the bract leaves ternate. *F. involucrata* comes from south-eastern France and has narrow leaves and capsules without wings. *F. ionica,* from the Ionian Islands and western central Balkan Peninsula, has broad leaves and capsules without wings; *F. pontica* from the central and eastern central Balkan Peninsula, has leaves of medium width (generally intermediate between those of *F. involucrata* and *F. ionica*) and capsules with wings. Relative to *F. pontica,* it would appear that *F. involucrata* and *F. ionica* have two mutational character differences—one in common and one each independent and morphologically at opposite ends of a series (leaf breadth). If the three be united as one species, *F. involucrata* is the earliest name. One may postulate *F. pontica* as the oldest taxon of the three. A mutation then gave non-winged capsules and other mutations differing in leaf breadth.

Comments may be made here on one other group which has many problems. This is that of the fritillaries of the Himalaya and China of the *F. cirrhosa*—*F. roylei* affinity. If these be united as one species the name *F. cirrhosa* has to be applied to it. Typical *F. cirrhosa* has cirrhose leaves and occurs in Nepal, Sikkim, Bhutan, southern Tibet and Upper Burma. *F. roylei* with broader non-cirrhose leaves is found in the northern Punjab, Hazara, Kashmir, Tehri State, northern United Provinces, and Simla. So far as plants of the Indian Himalaya are concerned there would appear to be two species (or subspecies) *F. cirrhosa* in the east and *F. roylei* in the west with their ranges meeting presumably somewhere in Nepal. However, there are complications when collections from Tibet, northern Assam, and China are examined. In these areas there are plants intermediate between *F. cirrhosa* and *F. roylei* and others that deviate from both and yet belong to and by intermediates are linked up with the group. Unfortunately it has not been possible to study collections of living plants of this group grown under controlled conditions and dried specimens often leave much to be desired. It can only be tentatively suggested that the populations of *F. cirrhosa* and *F. roylei* originated from a more varied population to the north of the Indian Himalaya and that their relative uniformity, which is only another phrase for high degree of correlation of characters, is due to isolation at the south western and south central margins of the range of the group as a whole. How best to treat the mass of variants in Tibet and China taxonomically is another problem still awaiting solution. It is interesting that well marked tessellation occurs in many of the Indian

and Chinese plants and some individuals are not so greatly dissimilar from our own snakesheads.

Reticulation between Fritillaria and Other Genera

We must now turn to a brief consideration of reticulation between *Fritillaria* and other genera and again it is convenient to select certain characters of organs as examples of problems of particular interest.

Bulbs. In *Fritillaria,* several bulb types are present. This is true also of *Lilium* and there are good reasons for accepting a genetic relationship between the two genera. The bulb type with many small scales found, for example, in *F. camschatcensis,* can be practically matched in *Lilium,* as for example in *L. grayi, L. roezlii,* and *L. occidentale.* These species would also connect up with the "rice-bulb" type of American fritillaries. Moreover, *Notholirion hyacinthinum* has structures at least superficially very like "rice-bulbs" though the detailed morphology and ontogeny of these requires investigation in all the genera mentioned.

Leaves. The various leaf arrangements in *Fritillaria* occur also in *Lilium.* Cirrhose leaves are limited to *Fritillaria* within the *Tulipeae.* Their occurrence in *Gloriosa* may be and in *Mutisia* (*Compositae*), etc. certainly is an example of false reticulation.

Tepals. Tessellations are peculiar to *Fritillaria* within the *Tulipeae* as also are fasciae. Perianth shapes from campanulate to widely open and with or without recurved tepals can be matched in *Lilium, Notholirion,* and/or *Nomocharis.*

Nectaries. The long narrow furrowed nectaries of some fritillaries occur also in *Korolkowia* and *Lilium* but the ovate to almost circular pit of some *Fritillaria* species is apparently restricted to this genus within the *Tulipeae.*

Androecium. Papillose and glabrous filaments occur in *Fritillaria,* as we have seen, and both these characters occur also in *Lilium* but *Nomocharis* species have all smooth filaments. It is interesting to note that the North American lilies have glabrous filaments in agreement with the glabrous filaments of the North American fritillaries.

Gynoecium. The style and stigma development is more variable in *Fritillaria* than in *Lilium.* The undivided stigma of some species of *Fritillaria* occurs in *Lilium,* the trifid stigma of other species reappears in *Notholirion.*

Capsules. Fruits with rounded or acutely ridged valves occur in *Lilium* as in *Fritillaria.* In a few species of *Lilium* and in *Nomocharis* there are narrow wings developed but, so far as material is available, it would seem that the more extremely winged condition is found only in some species of *Fritillaria.*

Conclusions

Enough has, it is hoped, been selected from the mass of data already available to indicate the problems of reticulation with which the taxonomist is confronted. If one set of characters, say those of the bulbs, or of the leaves, or of the tepals, or of the style, or of the capsules, be selected as of primary value a fairly definite but "artificial" classification can be made. If all characters are utilized and given equal value a natural classification should, in theory, be obtained, but it is in many places indefinite in that it is blurred by mixed combinations of characters. Such reticulation still needs much further detailed study and one can only suggest that the following possibilities for any concrete instance should be kept in mind:

1. The occurrence of simple parallel mutations resulting in homologous variation.

2. The flow of genes through hybridization of populations and even of taxonomic species.

3. The restrictive effects of natural selection of environments.

4. The restrictive effects of inheritance of genoms from an ancestral type a long way back.

5. The limitation imposed by the mechanics of structure.

EVOLUTION OF THE MECHANISM OF CASTE DETERMINATION IN THE GENUS *MELIPONA*

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INTRODUCTION

Social bees (*Apidae*) belong to three tribes and five genera: *Bombus* (*Bombini*), *Apis* (*Apini*) and *Trigona, Lestrimelitta* and *Melipona* (*Meliponini*). These three tribes are phylogenetically related to one another possessing certain characters in common, and thus are united in the same subfamily (Apinae). Their morphological relationships were studied by Michener (1944), who constructed from these studies, a phylogenetic tree, suggesting a common ancestor for the tribes of social bees. Recent work (Kerr, 1946) has disclosed the existence of two very distinct methods of determination of castes (queens or functional females on one hand, and workers or females with under-developed ovaries on the other). It was known for some time that in the honeybee (*Apis mellifera*) a larva develops either into a worker or into a queen depending upon the food on which it is fed. The difference between a worker and a queen is, thus, phenotypic or trophogenic. A situation similar in principle seems to obtain also in *Bombus* and in *Trigona* and *Lestrimelitta*. In *Melipona*, no difference is observed between the feeding of future queens and of future workers. *Melipona* queens are genetically different from *Melipona* workers, the former being heterozygous for two or for three (depending upon species) genes, homozygosis for any one of which makes a diploid individual a worker. Some consideration on the probable course of evolution of the caste-determining mechanisms are reported in the present article.

EVOLUTIONARY RELATIONSHIPS AMONG THE SOCIAL BEES

Paleontological evidence, as well as morphology and bionomy, indicate that the tribe Bombini is the most primitive among the three tribes of social bees. Six genera of *Bombini: Protobombus, Electrapis, Chalcobombus, Sophrobombus, Ctenoplectrella, Glyptapis* are known from Oligocene (Baltic) amber (Wheeler, 1928). No fossil *Apini* are known, but the genera *Electrapis* and *Protobombus* are regarded morphologically intermediate between *Bombus* and *Apis*. Three Miocene *Meliponini* are known: *Meliponorytes succini,* and *M. sicula* from Sicilian amber, and *M. devictus* from Burmese amber (Tosi 1896, Cockerell 1921).

The anatomical traits of the modern *Apini* and *Meliponini* may be derived from those of *Bombini*. Thus, *Bombus* has dorsal as well as ventral wax glands, and both females and males possess them. The *Meliponini* have only dorsal wax glands, and even these are present only in young workers and in drones. The *Apini* have only ventral wax glands, and these are restricted to the workers.

Bombus has 3 to 6 egg-strings in the ovaries and an equal number of seminal tubes in the testes (Bordas, 1895). *Apis* has about 180 egg strings and seminal tubes, which represents a modification induced by strong selection for high fecundity. The *Meliponini* have 4 egg-strings and seminal tubes. *Bombus* and *Apis* have an accessory gland in the male reproductive system which is absent in

the *Meliponini*. The sting is well developed in *Bombus* and *Apis* but it has suffered atrophy in the *Meliponini*.

External morphology also suggests that the *Bombini* are close to the ancestral stock of social bees (Michener, 1944). Thus, *Bombus* has bare eyes and cleft claws; *Apis* has pilose eyes and cleft claws, and the *Meliponini* have bare eyes, simple claws in females, and cleft ones in males.

Bombus stores honey and pollen in oval pots of cerumen (wax mixed with gum, resin, latex, etc.) and in old cocoons. Some European species, such as *B. pomorum*, keep pollen stocks in cylindrical tubes. *Apis* builds no pots and keeps honey and pollen in new or old wax cells. In the *Meliponini* honey and pollen are stored in cerumen pots but a group of *Trigona* species (such as *T. silvestrii*) uses for this purpose cylindrical tubes, like *Bombus pomorum* (Wheeler, 1928). The writer has observed two weak colonies of *Melipona marginata* and *Melipona schencki* which were exceptional in that brood cells were used for honey storage, instead of pots, as is normal. This is a link between the food storage systems used by the *Meliponini* and the *Apini*. The ancestors of *Apis* may have stored food in pots, as the *Bombini* and *Meliponini* do now.

The *Apini* and *Meliponini* have, however, some traits suggesting a common origin. Both have the same type of corbicula, no hind tibial spurs, and have well developed jugal lobes in their hind wings and labrum three to four times as broad as long (Michener, 1944). Both construct individual cells for the progeny, special queen cells (in *Apis, Trigona* and *Lestrimelitta*), perennial colonies (instead of annual ones as in *Bombus*), and practice individual feeding of larvae.

Caste Determination in the Bombini and Apini

In *Bombus*, no queens develop in young colonies. The first larvae raised by the founding female receive little food and develop into diminutive workers. Only when a colony reaches the stage of having numerous workers, does the food given to the brood become more abundant, and some fertile females begin to appear.

In *Apis dorsata*, the most primitive *Apis* species, drones and workers develop in similar cells (Singh, 1943). The domestic bee, *Apis mellifera*, raises workers in small and drones in larger hexagonal cells, while queens develop in peanut-shaped cells placed preferentially at the bottom of the comb. Larvae of queens are fed on a so-called "royal jelly," while worker and drone larvae receive pollen and honey from the third day of their life on (Snodgrass 1925). The well-known evidence of phenotypic caste determination in *Apis mellifera* is that when eggs originally placed by the bees in worker cells are transferred to queen cells, the larvae arising from these eggs develop into queens.

Caste Determination in the Meliponini

In the Meliponini larvae are raised in individual cells, as in *Apis*. Each cell is filled with honey, pollen and a glandular secretion. In most species, as soon as the queen lays an egg in a cell, the latter is sealed, to be opened again only by the emerging imago. In some species (such as *Trigona carbonaria*, Rayment 1932, 1935) the cells are however kept open for several days, new food being added even after the hatching of a larva. In either case, feeding is "progressive," *i.e.*, a young larva eats first the glandular secretion, then the honey, and lastly pollen. The system of larval feeding used by *Trigona carbonaria* resembles, therefore, that practiced for queen larvae of *Apis mellifera* (Kerr, 1948).

Trigona and *Lestrimelitta* have two kinds of cells: small ones for workers and drones and large ones for queens. The queen cells may be twice, or even more than twice, as large as the worker ones, according to species. This writer has established by statistical tests that

queen cells are placed mostly at the margins of horizontal combs. Although one can not be absolutely certain about this, all the available evidence indicates that the food placed in queen and in worker cells seems to differ only in quantity.

There can not be any doubt that caste determination in *Melipona* is not trophogenic, but genotypic. The evidence, given in more detail elsewhere (Kerr, 1946, 1948, 1950), can be summarized as follows:

a) There are no differences in the quantity of food provided for the larva, as can be concluded from the following facts: all cells are of the same size and contain the same amount of food, with an average weight of 148 mg. per cell. The food absorbed by both queen and worker larvae is the same, as shown by the identity of the mean weight of young queen and worker pupae. The only statistical difference noticeable is the higher variability of worker pupae, as measured by the standard error.

b) It seems impossible to assume that there exist any differences in quality of food, since queens' cells are distributed at random in the horizontal combs and are found in normal hives in fixed ratios.

c) Under unfavorable conditions, or in diseased hives there is a considerable reduction in the frequency of emerging queens, to one fourth or less of the expected ratio, which needs a special explanation. No elimination of eggs, larvae or pupae occurs, with individuals emerging from every one of the closed cells. There are no intermediates and queens are always of normal appearance and quite distinct from workers as verified in more than 50,000 individuals studied to date. If one wanted to refer to some trophogenic hypothesis to explain this situation, one would have to make the rather improbable assumptions: 1) that workers provide or omit completely a certain foodstuff necessary for queen formation, 2) that they deposit this stuff completely at random, 3) that they apply it to less and less cells under unfavorable conditions, never exceeding however a fixed maximum under favorable ones. This maximum is about 25 per cent in one species and about 12.5 per cent in seven other species.

There can be little doubt that the process which reduces the number of queens is a general cytogenetical phenomenon since it is not limited to the ratios of queens to workers but also applies to the segregation of the monofactorial difference: black against yellow first abdominal segments.

d) Thus, limiting the discussion to normal hives it was found that in *Melipona marginata* and its subspecies workers are about three times as numerous as queens (ratio 3:1). In other species so far studied in this respect, namely *Melipona quadrifasciata, Melipona schencki, Melipona favosa orbignyi, Melipona interrupta fasciculata, Melipona rufiventris paraensis, Melipona flavipennis,* and *Melipona fuscata melanoventer,* workers are seven times more numerous than queens (ratio 7:1).

The occurrence of the 3:1 ratio suggests that the queens are heterozygous for two pairs of genes (AaBb), homozygosis for either or both of which makes a female a worker (thus, workers are AABb, AaBB, aabb, aaBb, AABB, etc.) and males are AB, Ab, aB, or ab). In species from which the 7:1 ratio is characteristic, the queens are heterozygous for three pairs of genes (AaBbCc), while workers are homozygous for one, two, or three of these genes (AaBBCC, AaBbCC, AABBCC, etc.). It is easy to show that, with such a system, any mating of a queen with a haploid male (drone) results in production of a single genetic type of queens, several genetic types of workers, and several types of males (Kerr, 1946, 1950).

The Origin of the Genotypic Mechanism of Caste Determination

The phenotypic, or trophogenic, caste determination is presumed to be phylogenetically more ancient than the geno-

typic caste determination mechanism. The antiquity of the trophogenic mechanism is indicated by its occurrence in the genus *Bombus,* which, as shown above, must be regarded as primitive for a variety of reasons. Furthermore, this mechanism occurs in all three tribes of social bees, while the genotypic one exists only in a part of the tribe *Meliponini.*

The ancestors of *Melipona* probably had their castes determined trophogenically, as is the case at present in *Trigona* and *Lestrimelitta.* In such an ancestor, with a genetic constitution AABBCCDD . . . , a mutation took place from A to a, the heterozygote Aa being heterotic, thus possessing a higher adaptive value than the original genotype. Assuming that the drone which fertilized this Aa queen was A, AA and Aa queens and AA and Aa workers would be produced, depending upon the feeding of the larvae. The heterosis might, however, make Aa workers fertile, and not sterile as their AA sisters. The heterosis of the Aa gene combination would, then, lead to increase of the frequency of the new allele a in the population, up to the point when the disadvantages of homozygosis for A(AA) would be counterbalanced by the disadvantages of homozygosis for a (aa). Modern genetics demonstrates many situations in which both homozygotes for a gene or a chromosome structure have adaptative values inferior to the corresponding heterozygote (Wright and Dobzhansky, 1946).

A new mutation, B to b, has then happened at another locus, having the same heterotic properties as the A locus, females Bb will, then, have an advantage over BB and bb. Heterozygosis for both genes, AaBb, might confer so great an increase of vigor on the double heterozygote that the latter might develop into a queen regardless of kind and quantity of food which it received in the larval stage. This would make the trophogenic mechanism superfluous, and all the homozygotes would develop automatically into workers. Owing to the existence of fertile heterozygous females in every colony, mutations that cause loss of the instincts connected with the construction of distinct royal and worker cells would now be tolerated instead of being eliminated by natural selection, as would occur in a species with trophogenic caste determination. *Melipona marginata* and its subspecies may be regarded as representing the most primitive now existent type of genotypic caste determination among bees.

The transition from the bifactorial (AaBb) to the trifactorial (AaBbCc) type of caste determination could occur by addition of a third mutation, C to c, with heterotic properties. The triple heterozygotes, having selective advantages thus became established as the exclusive genotype in queens, while the AaBbCC and AaBbcc type became a worker. With a trifactorial mechanism a decrease in the proportion of queens in colonies would occur (1:7 instead of 1:3 ratio). Indeed, with the bifactorial mechanism the queens constitute 25 per cent of all diploid females, while with the trifactorial one only 12.5 per cent of the females are queens, the remaining 87.5 per cent being workers. This may have an important adaptative advantage. Most virgin queens appearing in a *Melipona* colony are killed off by the workers within 15 days after emergence (Kerr, 1950). This means that with the bifactorial mechanism, twice as many queens will be eliminated as with the trifactorial mechanism. The lesser "cost" of the trifactorial, compared to the bifactorial mechanism may explain why a majority of species of *Melipona* studied in this respect have the former, and only one species, *M. marginata,* still preserves the latter type of mechanism. The genes involved in bifactorial as well as in the trifactorial mechanism have a sex-limited action. Males, despite being homogametic, remain fertile. The abundance of genes with sex-limited effects in *Hymenoptera* is quite understandable. As expressed by White (1945, pp. 279): "Recessive mutants that are sex-limited in their expression so that they produce

no effect in the male, will be in special position in organisms with male haploidy. Such mutations may exist in wild populations for a long while, even though deleterious, since in the males they will not affect the phenotype. This may possibly explain why in the social *Hymenoptera* the males are all of one type while the females are differentiated into several castes. We are not suggesting here that the various female castes are genotypically different—merely that a large number of mutants whose effects are limited to the female sex may have accumulated in the species, gradually building up a genotype that is very plastic in the female sex (producing several entirely different phenotypes according to the environment and nutrition of the larvae)."

Genotypic mechanisms of caste determination have, however, certain adaptive disadvantages. The reproductive wastage involved in killing off a part of the queens emerging in the colony has already been mentioned. Anyway, a rigid mechanism which produces a fixed proportion of queens and workers may be less desirable than a mechanism which allows a greater plasticity in this respect. It appears that, superimposed on the genotypic caste determination, the genus *Melipona* has evolved another mechanism which permits variation in the proportion of queens to workers among the developing larvae. During winter, or in colonies with few nurse bees, or in hives infected with parasites or diseases, the percentages of queens among the hatching brood may be very small or even zero. The precise nature of this additional mechanism has not been completely clarified. We have mentioned in a general way the reasons why we do not think that any phenotypic or trophogenic mechanism can be responsible. Thus we have arrived by exclusion of other possibilities to the following hypothesis which fits all data so far on hand. If there were a parthenogenetical development of eggs with a following fusion of two nuclei of identical constitution, either derived from an equational meiotic division or from an early cleavage division, we would obtain diploid homozygous individuals. In accordance with our theory of caste determination, these must be all workers. A somewhat analogous reduction is known in the fish *Lebistes reticulatus* in which Winge (1934) found the sex-ratio to vary greatly with season. In spring a normal sex-ratio of 1 ♀ : 1 ♂ is produced, while at other times most of the young are females.

Discussion

According to the hypothesis presented above, the transition from the trophogenic to the genotypic mechanism of caste determination entails production of mutations (A to a, B to b, C to c) with heterotic effects in heterozygotes (Aa, Bb, Cc) and deleterious ones in homozygotes (AA, aa, BB, bb, CC, cc). Such heterotic effects are indeed possible in organisms with the haplo-diploid sex- determination, characteristic for *Hymenoptera*. Mackensen (in Farrar, 1943–1947, and Farrar, 1948) obtained offspring from honeybee queens fertilized by their own sons. Such an inbreeding process results in a rapid loss of vigor of the colony and, particularly, in a loss of ability of the eggs to hatch. Intercrossing of inbred lines gives, on the contrary, a pronounced hybrid vigor. Some double-hybrid colonies were highly productive, but their worker bees were "intolerably vicious." This latter quality may perhaps be regarded as an additional manifestation of heterosis. The genetic mechanism involved in production of heterosis evidently must involve genes that are advantageous when heterozygous in diploid individuals (Aa superior to both AA and aa) but which do not affect adversely the haploid individuals, A and a. Both, increase and loss of vigor in *Apis* have not yet been studied genetically, but it seems to be of the same nature as this phenomenon in maize. In this species recent studies by Crow (1948) and Brieger (1948, 1949) have shown that the heterotic mechanism can be attributed only to a small extent

to the combination of dominant factors, being mainly due to the interaction of heterotic genes in the heterozygous conditions. Our Mendelian formula applies the same genetical principle of a heterotic gene interaction for caste determination. Individuals completely heterozygous are fully fertile (queens), while partially or fully homozygous individuals are sterile (workers).

The type of sex-determination discovered by Whiting (1940) in *Habrobracon juglandis* may be regarded as involving a special type of heterosis. Here, a diploid individual must, in order to be a female, be heterozygous for different alleles (or chromosome sections). Homozygosis for either allele gives a poorly viable diploid male, but the haploid males carrying either allele are fully vigorous. In *Melipona,* the heterosis is exploited for purposes of caste rather than sex-determination. And furthermore, since the caste-determining genes (A, B, C) are localized in different chromosomes, any queen is automatically heterozygous for three chromosome segments, in which further heterotic genes may accumulate.

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Summary

Two types of mechanism of caste determination exist in social bees. In the tribes *Bombini, Apini,* and in a part of the *Meliponini* (genera *Trigona* and *Lestrimelitta*), a diploid individual develops into a fertile female (queen) or into sterile female (worker) depending upon the quality and quantity of food which it receives in the larval stage. This is the phenotypic, or trophogenic, caste determination. But in the genus *Melipona,* the caste determination is genotypic. Queens are heterozygous for two (*Melipona marginata*) or three (all other species of *Melipona* so far studied) genes, each of which, when homozygous, makes the individual a worker.

Among the social bees, the trophogenic mechanism of caste-determination is phylogenetically primitive and the genotypic mechanism secondary. The former mechanism exists in every one of the three tribes of social bees, and is the only one in the tribe *Bombini,* which must be regarded as primitive on several grounds. The genotypic mechanism is known only in the specialized genus *Melipona.*

The emergence of the genotypic mechanism in the ancestors of *Melipona* which are presumed to have had their castes determined phenotypically, is visualized as a succession of mutations in genes with sex-limited heterotic effects. The transition from the genotypic mechanism involving two genes to one involving three genes had an adaptative advantage because of the decrease of the proportion of the obligatorily produced queens. An additional mechanism, which permits variations in the proportions of queens born in a colony at different seasons, has become superimposed on the genotypic caste determination in *Melipona.*

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EXPERIMENTAL POPULATIONS OF *DROSOPHILA PERSIMILIS* FROM AN ALTITUDINAL TRANSECT OF THE SIERRA NEVADA[1]

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INTRODUCTION

Populations of *Drosophila pseudoobscura* and *D. persimilis* throughout their respective distribution areas in western North America exhibit a very extensive and intricate pattern of gradients, or clines, in relative frequencies of the gene arrangements of the third chromosome (Dobzhansky and Epling, 1944). These gradients may be very gradual over a wide area from north to south or west to east, or they may be rather steep in slope across an altitudinal transect of relatively short distance in a mountain range (Dobzhansky, 1948a).

Within certain populations of *D. pseudoobscura* temporal fluctuations of less amplitude than the more extensive geographic differences have been observed (Dobzhansky, 1943, 1947a) while in some other populations of the same species long term trends were found (Dobzhansky, 1947b). It was by studying the behavior of these chromosomal types in artificial populations (Dobzhansky, 1947a, Wright and Dobzhansky, 1946) and later by statistical analysis of wild populations (Dobzhansky and Levene, 1948) that these gene arrangements were proved to have selective values and were shown to be maintained at the observed frequencies by natural selection under both wild and artificial conditions. This information together with evidence of physiological differences between carriers of each arrangement (Heuts, 1947b, 1948) leaves no doubt as to the importance of variation in gene arrangement in the process of *Drosophila* species' adaptation.

However, no well-established seasonal cycles or long term changes have been observed as yet in any population of *D. persimilis*. It is of interest, therefore, to test the gene arrangements of this species and to compare its adaptive chromosomal mechanisms with those of its very close relative, *D. pseudoobscura*.

The summaries of literature on clines by Mayr (1942) and by Huxley (1943) indicate the vast amount of observation which has been made through morphological taxonomic studies of polymorphic species; but the number of such investigations which have dealt with the genetic aspects of the cline are disappointingly small. It may be of interest to list a few of the more recent works pertinent to this report:

(1) Perhaps the most thorough of recent studies concerned with the genetic differences between local populations from continuously inhabited areas is that of Clausen, Keck, and Hiesey (1940, 1948) for various plant species in western United States.

(2) Dubinin and Tiniakow (1947) discovered an interesting gradient in inversion frequencies of *D. funebris* throughout European Russia. No studies were made on gene contents of these inversions from different localities (Dubinin and Tiniakow, 1946a, b).

(3) Reed, Williams, and Chadwick (1942) studied wing-beat frequency and several thoracic and wing dimensions in various species of *Drosophila*. Attention should be called to the gradients of wing-beat frequency among the related species: *D. pseudoobscura, D. persimilis,* and *D.*

[1] The present paper is a revision of a thesis submitted to the Department of Biology of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1949.

miranda. Reed and Reed (1948) investigated further the morphological differences between *D. pseudoobscura* and *D. persimilis*. Not only were some interesting gradients established but it was pointed out that strains from each locality were often statistically distinctive.

(4) Carson and Stalker (1947a) described nineteen different gene arrangements in *D. robusta* of which fifteen are widespread and distributed in striking gradients from north to south and west to east. Later these authors (1947b) gave an extensive analysis of morphological clines of various dimensions: length of thorax, width of head, length of fore-femur, and length of wing. They pointed out the parallel between these cytological and morphological gradients.

Later a very intensive study of morphological and cytological gradients in *D. robusta* was made on an altitudinal transect in the Appalachians of Tennessee (Stalker and Carson, 1948). This investigation revealed two important facts pertinent to this discussion: (a) Some of these gradients paralleled their more extensive north-south counterparts, but there were some notable inconsistencies between the altitudinal and geographical clines. (b) More pronounced changes in frequency of certain inversions took place above 2,000 and below 1,400 feet; in addition, the greatest morphological changes were found in that very area between 2,000 and 1,400 feet. The authors concluded that this partial discontinuity was evidence of the "northernness" of the upper population and the "southernness" of the lower. Both this evidence of discontinuity within an apparently continuous cline and the problem of discrepancy between the geographical and altitudinal inversion gradients will be discussed later at some length in this report. It should be stressed here, however, that the words "northernness" and "southernness" are ambiguous and refer only to parallel morphology and not to any basic similarity between the gene contents of any inversion from high altitude and northern United States, for example. Such probabilities or improbabilities are to be discussed after the experimental evidence from *D. persimilis* has been considered.

The Problem

Geographic and Altitudinal Gradients in Relative Frequency of Gene Arrangements of Drosophila persimilis

The present study is concerned with an altitudinal gradient in the relative frequency of four gene arrangements in the third chromosome of *Drosophila persimilis* along an east-west transect on the western slope of the Sierra Nevada of California first described by Dobzhansky (1948a). The purpose is to investigate the nature of this gradient through an analysis of laboratory populations which are composed of flies descended from wild parents collected at opposite ends of the altitudinal transect.

D. persimilis has been collected from British Columbia to Santa Barbara County, California, and from the Pacific eastward to the Cascades and Sierra Nevada. Within this area it is most common in the cooler and more humid habitats, especially the coast north of San Francisco and the western slopes of the mountain ranges where it is characteristically an inhabitant of forests at higher elevations.

Of the ten known gene arrangements of the third chromosome in this species, four are well established throughout most of the distribution area. Of the remaining six, three are scattered at low percentages in a few localities, one (Mather) was recently described by Dobzhansky (1948a), and two are described in this report for the first time. The standard map of the third chromosome and descriptions of most of the known inversions were given by Dobzhansky and Sturtevant (1938); later revisions and additions to the data were given by Dobzhansky and Epling (1944).

The standard arrangement (ST) has four times given rise to different inver-

sions in *D. persimilis* by one change in the sequence; of these the Klamath (KL) (one fifth total length of the chromosome) and the Mendocino (MD) (one third length of chromosome) arrangements are common; but the Whitney (WT) (one fourth length of chromosome) arrangement, a single step derivative of Klamath, is by all means the dominating inversion in the area of the altitudinal transect under consideration. KL is the most widely distributed of the four. It is common in British Columbia and the Coastal and Cascade Ranges of Washington where populations often reach nearly 100 per cent KL. As one goes southward on the coast MD becomes increasingly common until it reaches a maximum frequency of 69.6 per cent at Cape Mendocino, California; also MD forms a gradient from the Pacific coast eastward. In the southern Cascades and northern Sierra Nevada KL falls sharply, and WT becomes more prominent; finally in the Mt. Whitney region WT reaches a maximum of 90 per cent frequency. ST is a more or less filler-in arrangement; it is never the most frequent even where it attains its maximum frequency of 33 per cent in Sequoia Park, California.

Dobzhansky (1948a) has not been able to find any positive evidence for cyclic seasonal changes in relative frequencies of these inversions in any locality which would be comparable to the changes found in *D. pseudoobscura* (Dobzhansky, 1943, 1947a, 1947b; Wright and Dobzhansky, 1946). In many cases, however, his individual samples of *D. persimilis* are not large enough to treat statistically; and it may very well be that in the future some seasonal cycles in this species will be found, especially at lower elevations where the breeding season is comparatively long. Because it has been shown that these seasonal cycles are maintained by natural selection (Dobzhansky and Levene, 1948) the question arises as to whether altitudinal gradients in relative frequencies of inversions are also maintained by natural selection, and if so, whether the gene arrangements manifest differences in adaptive values under laboratory conditions.

Descriptions of collecting areas and

TABLE 1. *West-east transect collections of* D. persimilis *showing relative frequencies of gene arrangements in per cent for seasons from 1946 to 1948 (data from Dobzhansky 1948)*

Locality	1946					1947					1948 (February)				
	Sample size	WT	ST	KL	MD	Sample size	WT	ST	KL	MD	Sample size	WT	ST	KL	MD
Upper Sonoran Life Zone															
Jacksonville 850′	2	100	0	0	0	58	58.5	10.3	12.1	19.1	172	56.4	9.3	23.3	11.1
Transition Life Zone															
Lost Claim 3,000′	30	63.4	3.3	23.3	10.0	142	81.7	12.0	4.2	2.1					
Mather 4,600′	128	77.0	11.0	10.0	2.0	432	79.9	8.3	6.9	4.9					
Aspen 6,200′	108	68.5	11.1	14.8	5.6	262	81.4	9.2	5.4	4.0					
Pate 4,200′	0	—	—	—	—	60	76.4	13.4	5.0	5.0					
Canadian Life Zone															
Porcupine 8,000′	82	76.8	11.0	7.3	4.9	120	85.8	9.2	2.5	2.5					
Benson 8,000′	0	—	—	—	—	110	88.2	9.1	2.7	0.0					
Tuolumne 8,600′	80	92.5	5.0	2.5	0.0	274	90.5	4.0	1.8	3.7					
Hudsonian Life Zone															
Timberline 9,900′	30	93.3	0.0	3.3	3.3	44	90.9	6.8	0.0	2.3					
Lyell Base 10,500′	0	—	—	—	—	58	87.9	8.6	3.4	0.0					

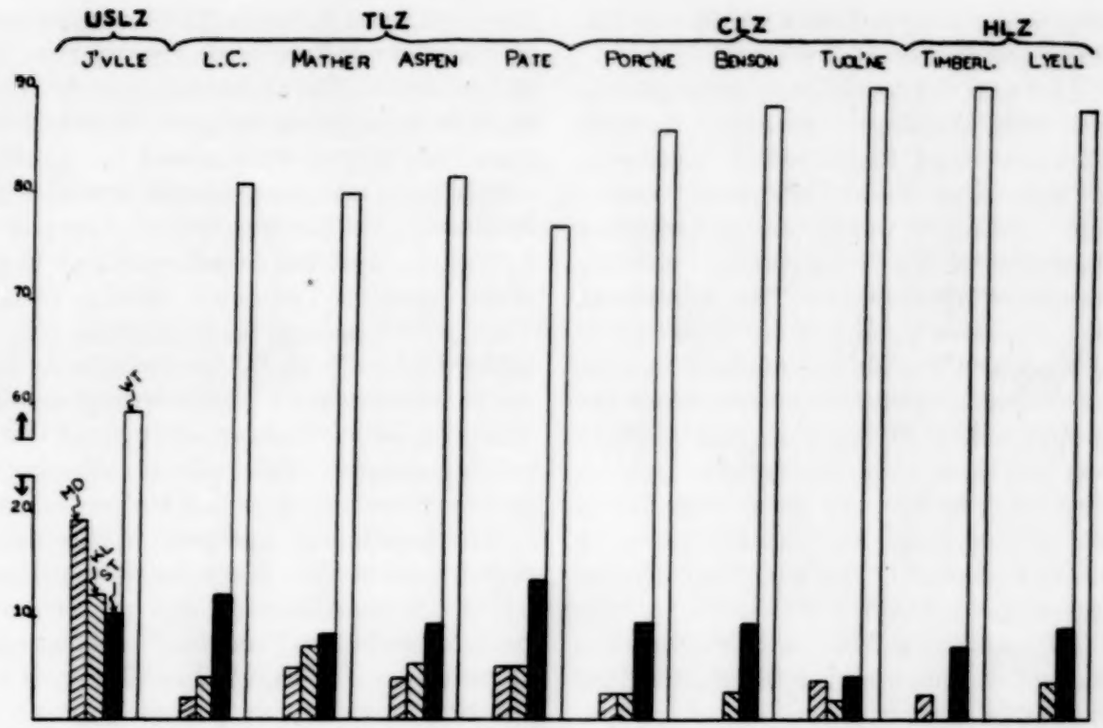

FIG. 1. Graph of the relative frequencies of the gene arrangements in *D. persimilis* according to the 1947 collecting data (Dobzhansky, 1948a). The localities are arranged according to their sequence with increasing elevation from left to right (see table 1) and are grouped into Life Zones: Upper Sonoran (U. S. L. Z.), Transition (T. L. Z.), Canadian (C. L. Z.), and Hudsonian (H. L. Z.). The four inversions shown for each locality are MD, KL, ST, and WT as indicated with arrows under Jacksonville.

data for the gradient are given by Dobzhansky (1948a); however, for the sake of convenience and for additional discussion the data have been reorganized in table 1 so that 1946 and 1947 data could be treated separately. Figure 1 shows a graph of the 1947 collecting data alone since these data are the most extensive samples from the area.

Four important points relevant to this report are to be noted: (1) increase in WT with increase in altitude; (2) decrease of KL and MD with increase in altitude; (3) slight decrease, or relative stability, of ST (it is also interesting to note that ST is the least prominent of the four well-established inversions throughout the distribution area); and (4) a slight discontinuity evident on the fringes of the life zones (see fig. 1); that is, there is relative homogeneity within life zones but a slight sudden change between populations on the edges of the life zones, a fact which will be treated further in the discussion.

The Population Cage Approach

Experimental populations in cages have been used by Dobzhansky (1947a) to demonstrate "the postulated high selective advantages or disadvantages of the carriers of different gene arrangements in different environments." These selective advantages or disadvantages are presumably acquired by local populations in the process of evolution over many generations; and while it is obvious that the environment prevailing in artificial laboratory populations will by no means be identical with that in natural habitats, still many questions regarding the comparative behavior and consequently the relative selective values of the different gene arrangements can be answered with some accuracy.

The population cages in the present

study were designed to answer the following questions:

(1) Does the same gene arrangement from two strikingly different habitats (Tuolumne and Jacksonville) behave in the same way under laboratory conditions? In other words, are the adaptive properties of an arrangement uniform throughout this area? The answer to this question is important for understanding whether the observed gradient in relative frequencies results merely from the selective action of the ecological gradient upon the *same* gene contents in each locality or whether the gene contents of each arrangement are molded more or less independently in each locality, as postulated by Mayr (1945).

(2) Can any indication of mechanisms involved in maintaining this altitudinal gradient be determined by exposing population cages to different temperatures? Can it be said that the difference in pattern of frequencies at the two localities is determined in any way by the difference in the average temperature of the two localities?

Materials and Techniques

The flies for the Tuolumne cages were collected in August, 1947, at Tuolumne Meadows (el. 8,600′) and at Aspen Valley (el. 6,200′). These localities are about twenty miles apart; the Tuolumne collecting area is a forest near Fairview Dome of Yosemite National Park in the Canadian Life Zone; Aspen is a luxuriant forest in the upper Transition Life Zone near the Aspen Valley Ranger Station. Tuolumne is twenty-five miles east of Mather, California, while Aspen is about six miles southeast of Mather. (For annual temperature and precipitation data of this region see appendix.)

The flies for the Jacksonville cages were collected by Carl Epling in February, 1948, at Jacksonville (el. 836′) about twenty-five miles west of Aspen in an oak-pine grove of the Upper Sonoran Life Zone. At Jacksonville both *D. pseudoobscura* and *D. persimilis* are found, but the former is much the more common in summer while the reverse is true in the winter. The breeding season here extends throughout the year. At Mather, about half-way up the transect (el. 4,600′) both species are about equally common in summer. Mather lies in the Transition Life Zone, and the breeding season lasts from April to October. In the Upper Transition (Aspen) and Canadian (Tuolumne) Life Zones *D. persimilis* is by far the more common. The breeding season, however, is very short, only from June to September. This period allows for only two to four generations per season.

The population cage used in this study was fundamentally the same as the original L'Heritier-Teissier box for Drosophila populations modified further by Dobzhansky (1946, 1947). There is no need to describe this population cage in any detail, but it should suffice to point out that the important feature of the cage lies in the fact that a continuous population can be sustained by adding new food medium and removing worked out food cups at definite intervals. The cages were kept in constant temperature rooms at 25 ± 0.5° C. and 16 ± 0.5° C.

Each food cup was kept moist by daily adding of yeast suspension until larvae approached maturity, at which time the cup was allowed to go dry while pupae were forming and thereafter. Cold room (16°) populations were far more crowded, and at an early date it was decided to insert rolled up strips of paper towelling into the cups of all cold room cages. This practice prevented drowning of adults in the food to a large extent as well as giving an excellent support for pupae. The population in cage no. 1 at 16° where paper towelling was first used about doubled from approximately twenty-five hundred to five thousand flies in one generation.

The technique of sampling used was that described by Wright and Dobzhansky (1946). Samples of eggs were taken each generation (every twenty-five days at 25° and forty days at 16°). Each

sample consisted of six subsamples taken in daily succession: a portion of food covered with eggs was cut out and placed in a culture bottle; larvae were matured at 25° C.; salivary glands were dissected and stained with acetoorcein from twenty-five larvae in each subsample, or a total of 150 larvae (300 chromosomes) for the entire sample.

For cages No. 1–No. 3 and No. 9 the initial population of flies was obtained by single pair matings within each strain. After four or five days a control, or check, count was made to determine more accurately the initial frequency of inversions. However, for cages No. 4–No. 8 stocks were maintained and checked by single pair matings for at least two generations before the cage was started. In these cases mass matings of stocks were usually made to obtain large initial numbers, but the constitution of the population was accurately known only after the control count had been made.

While it was important to have as many different strains as possible in one cage to provide for genetic heterogeneity but cytological homogeneity, unfortunately not very many strains of certain arrangements were available. This was the case with Tuolumne KL especially, and consequently cage No. 9 is to be considered only as it may illustrate principles derived from the other cages and not as an accurate measure of reaction for the particular arrangement from that locality. It should be noted that with only three strains of any arrangement opposed by three strains of another arrangement in any cage (a minimum of six different chromosomes involved) the total different zygotes possible would be fifteen; while with four strains of each chromosome the number of zygotic combinations possible would be twenty-eight, nearly twice as great. The number of strains involved in each cage, initial frequencies, and other pertinent initial cage data are given in table 2. (Relative humidity data for each month are given in the appendix. Zygotic data have not been included because of their excessive bulk.) Each cage contained WT chromosomes in competition with one other arrangement from the same locality.

The Population Cage Observations

Tuolumne Cages

Population cage No. 1 containing WT and MD chromosomes from Tuolumne and Aspen was placed first in the 25° constant temperature room for two generations in order to ascertain any changes in relative frequency. At the end of the two generation period no significant change in frequency had taken place. The results of this cage are given in table 3. There was a possibility, however, that the relative frequencies of the gene ar-

TABLE 2. *Initial data in the experimental populations*

Cage no.	Origin of chromosomes	Date started	Temperature	Initial number flies	Number of strains			
					WT	KL	MD	ST
1	Tuolumne and Aspen	Mar. 13, 1948	Both 25° and 16°	520	4	—	4	—
2	Tuolumne and Aspen	Apr. 20, 1948	Both 25° and 16°	500	4	—	—	4
3	Jacksonville	May 18, 1948	Both 25° and 16°	578	6	6	—	—
4	Jacksonville	Aug. 25, 1948	Both 25° and 16°	736	8	—	—	5
5	Jacksonville	Sept. 8, 1948 (discont.) repeated Dec. 10, 1948	Both 25° and 16°	1052	8	4	—	—
6	Jacksonville	Aug. 30, 1948	Both 25° and 16°	728	6	—	4	—
7	Jacksonville	Nov. 16, 1948	25° only	411	3	—	3	—
8	Jacksonville	Nov. 17, 1948	25° only	632	5	—	—	5
9	Tuolumne	Dec. 20, 1948	Both 25° and 16°	870	3	3	—	—

TABLE 3. *Relative frequencies of gene arrangements in cage No. 1 (Tuolumne and Aspen)*

Temp.	Date	% WT	% MD	Chromosomes examined
25° C.				
	Mar. 13, 1948			
	Initial	41.6	58.4	
	Control	41.1	58.9	304
	Apr. 13	41.5	58.5	304
	May 14	40.3	59.7	298
Transferred to 16° on May 27				
16° C.				
	June 18	49.0	51.0	302
	Sept. 8	58.3	41.7	290
	Oct. 21	58.0	42.0	300
	Dec. 10	56.0	44.0	300
Transferred to 25° on Dec. 30				
25° C.				
	Jan. 26, 1949	58.0	42.0	300
	Feb. 20	58.8	41.2	250

χ^2 (zygotic data at 25°) = 0.87, d.f. = 4, p. = 0.92.

χ^2 (zygotic data at 16°) = 28.98, d.f. = 6, p. < 0.01.

rangements in the initial population might have been by chance the equilibrium frequency which would be maintained by selection. Also it was desirable to know whether this same condition of no change continued at cooler temperature.

In order to answer these questions cage No. 1 was transferred to 16° C. Immediately a significant change in frequency took place: WT increased nearly 20 per cent with consequent drop in MD over a period of three generations, and an equilibrium was reached at approximately 58 per cent WT and 42 per cent MD. This cage was left at 16° for two additional generations as a check on the stability of the recorded proportions. The changes were highly significant. The cage was then transferred back to the 25° room.

If selection had been acting to maintain an equilibrium of 40 per cent WT — 60 per cent MD at 25°, then transferring the cage back to 25° would result in a return to that equilibrium from the condition observed at 16°. However, after two generations at 25° no evidence of change appeared. Such a result confirms the original assumption that the lack of change at 25° is due to absence of any significant selection pressure. (It is assumed that genetic drift is not effective enough to bring about any significant changes in inversion frequencies since the size of the population is constantly more than 1,500 individuals.)

It will be of interest at this point to discuss the relative adaptive values of the gene arrangements at 16° for comparison with later data. Sewall Wright (Wright and Dobzhansky, 1946) has given a general method for calculation of selective values of the three zygotic classes in a population containing two different gene arrangements (or alleles) in the same chromosome. This method, iteration by least squares,* was used by Dobzhansky to calculate the relative values of various gene arrangements in experimental populations of *D. pseudoobscura* (1947d and 1948b). The results of these

* If the survival rates of WT/WT, WT/MD, and MD/MD are termed W_1, W_2, and W_3, the proportions of the different genotypes following the stage of selective elimination would be as follows:

$$\text{WT/WT} = q^2W_1$$
$$\text{WT/MD} = 2q(1-q)W_2$$
$$\text{MD/MD} = (1-q)^2W_3$$

However, because the expression for Δq in terms of q (frequency) is not linear with respect to the selection coefficients, the solution of the formula given by Wright must be determined by iteration.

For each zygotic class the selective coefficients are symbolized as follows (same as Wright):

W = the adaptive value.
s = the selective disadvantage of WT/WT ($= 1 - W_1$).
t = the selective disadvantage of MD/MD ($= 1 - W_3$).
$\hat{q}$ = the frequency of WT chromosomes at equilibrium maintained by selection.

In the following calculations the adaptive value of the heterozygotes (W_2) is always equal to 1.00.

calculations for the changes occurring at 16° are as follows:

Genotype	W		
WT/WT	0.436	$s=0.564$	
WT/MD	1.000		$\hat{q}=57.2$ per cent
MD/MD	0.246	$t=0.754$	

Of course it should be noted that the change in temperature (or some accompanying change in humidity or both) has produced a measurable difference in adaptive values between the three zygotic classes; whereas at 25° there is no measurable difference between the classes ($W = 1.0$ for all classes).

Cage No. 2 containing WT and ST chromosomes from Tuolumne and Aspen showed no significant changes after two generations at 25° (see table 4), and for three additional generations at 16° no changes occurred. Apparently whatever agent or agents there may be which bring about differential survival of WT and MD zygotes at 16° from this locality, that agent has no effect on WT and ST at 16°. It is important to note, however, that so far no changes in frequency have been observed at 25° for any gene arrangements from these localities.

Cage No. 9 containing WT and KL chromosomes from Tuolumne was started after most of the Jacksonville cages had been completed. It has been noted above that only three strains of KL were available from this locality, and because of this small number of strains no experiment involving these strains was originally planned. However, after the results of Jacksonville WT–KL cages had been obtained, it was decided to start cage No. 9 merely for the sake of completeness. The data for this cage are given in table 4.

Cages No. 1 and No. 2 did not show a very pronounced increase in numbers of individuals in the 25° room over the initial number. As a matter of fact, a rough approximation of the number in these cages would be 1,000–1,500. This number increased between two and three fold after one generation at 16°. However, cage No. 9 did especially poorly at 25°; although the initial number was 870, no increase in number took place. In fact, it was maintained with some difficulty at the end of one generation. Few larvae seemed to reach pupation, and before any dangerous reduction of the population size might take place the cage was moved to 16°. Here at the end of another generation the number of flies was as great as in the other cages. It should also be mentioned here that it was much more

TABLE 4. *Relative frequencies of gene arrangements in cages No. 2 (above: Tuolumne and Aspen) and No. 9 (below: Tuolumne)*

Temp.	Date	% WT	% ST	Chromosomes examined
25°				
	April 20, 1948			
	Initial	52.0	48.0	
	Control	55.0	45.0	290
	May 21	54.9	45.1	306
	June 18	52.7	47.3	294
	Transferred to 16° on June 25			
16° C.				
	July 26	51.0	49.0	298
	Sept. 8	50.5	49.5	302
	Oct. 21	52.0	48.0	298

χ^2 (zygotic data both 16° and 25°) = 9.45, d.f. = 10, p. = 0.48.

Temp.	Date	% WT	% KL	Chromosomes examined
25°				
	Dec. 20, 1948			
	Initial	43.5	56.5	
	Control	40.4	59.6	250
	Jan. 30, 1949	42.6	57.4	230
	Transferred to 16° on Feb. 10, 1949			
16° C.				
	March 11	46.0	54.0	300
	Apr. 12	51.6	48.4	240

χ^2 (zygotic data at 25°) = 1.543, d.f. = 2, p. = 0.48.

χ^2 (zygotic data at 16°) = 7.94, d.f. = 4, p. = 0.092.

difficult raising larvae to maturity for cytological analysis from this cage than from any other. (The strains of WT used in cage No. 9 were different from those of No. 1 and No. 2 which in turn differed in strains of WT from each other.)

Unfortunately only one generation was counted at 25° for cage No. 9, but the frequency was not significantly changed from the initial frequency. Sampling for two generations after transferring to 16° showed an 8 per cent increase in WT at the expense of KL. While it is not possible to calculate an exact value for the selection coefficients on the basis of these data, it is apparent that WT is better than KL under the laboratory conditions at 16°. The changes which have occurred are only slightly significant, and while there is no overwhelming necessity for believing that actual changes have occurred, still on the basis of results from other cages it seems reasonable that the trend showing increase in WT and decrease in KL is a real one.

New Inversions in Tuolumne Strains

1.) An inversion not previously known was first found in population cage No. 2 during the control count (April, 1948). It is included within the WT arrangement and is about 12.5 per cent of the total chromosome length. The sequence of the bands (see original map of Dobzhansky and Sturtevant, 1938) and a drawing of a heterozygote of this inversion with ST are included in figure 2. In the control count 9 WT chromosomes were found to be heterozygous for this inversion (10.2 per cent of the WT/WT chromosomes or about 3 per cent in the entire sample). Throughout the sampling of cage No. 2 at least one heterozygote was found in each sample; this inversion was counted as a WT chromosome.

By a reexamination of the strains which had contributed to population cage No. 2, the inversion was rediscovered in strain No. 7 which is descended from a single wild female of Tuolumne. The chromo-

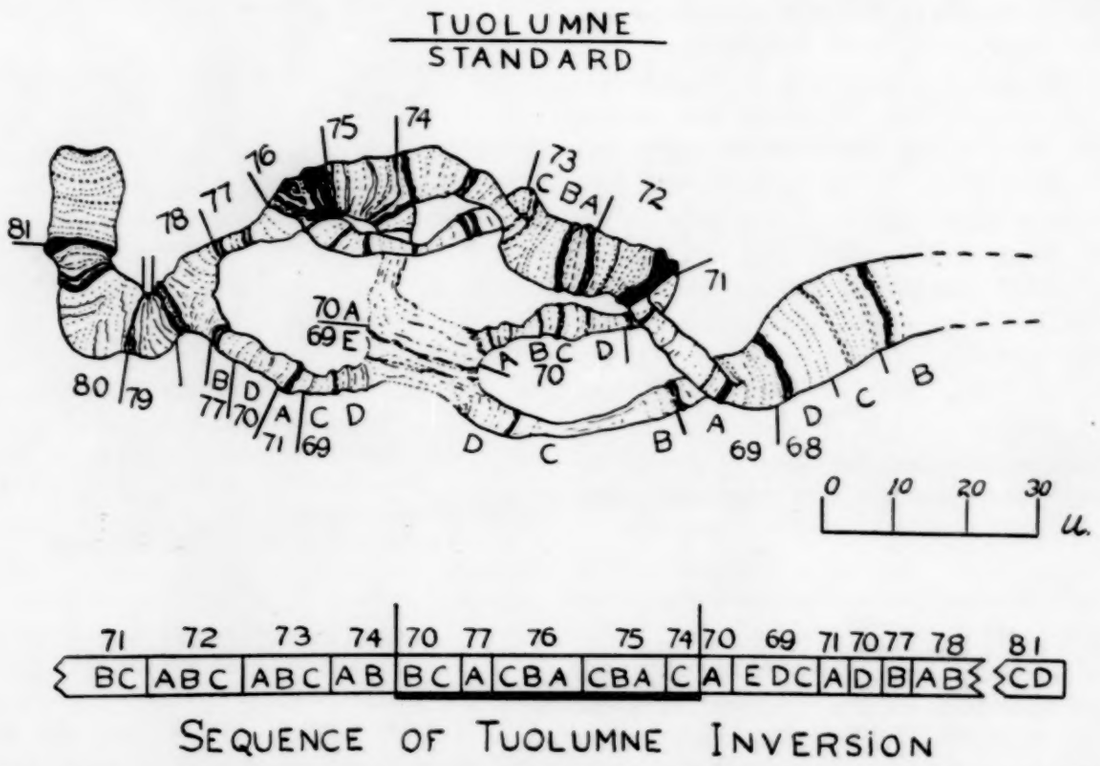

FIG. 2. The structural heterozygote of TU/ST. The Tuolumne arrangement is a small included inversion within WT. See text.

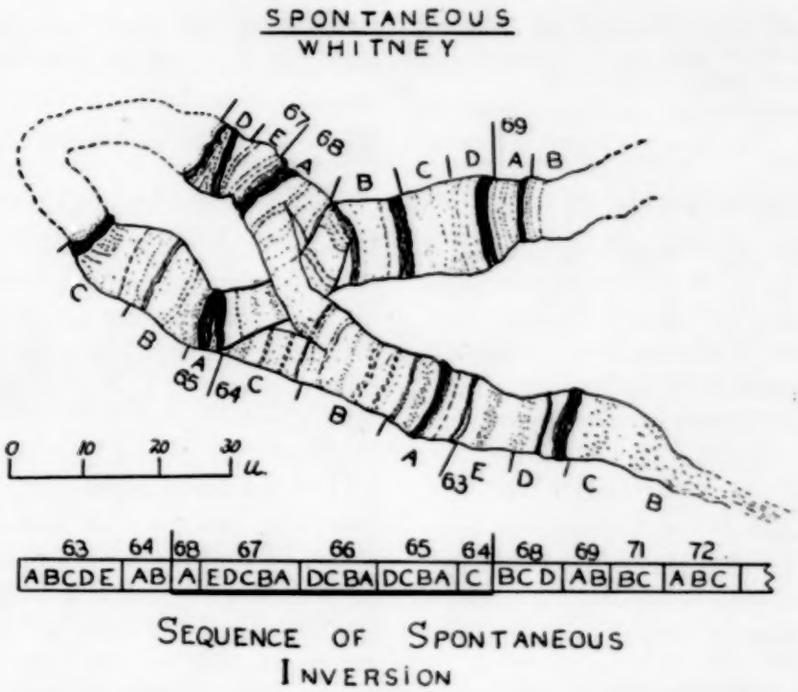
SPONTANEOUS
WHITNEY
0 10 20 30 u.
63 64 68 67 66 65 64 68 69 71 72
ABCDE AB A EDCBA DCBA DCBA C BCD AB BC ABC
SEQUENCE OF SPONTANEOUS
INVERSION

FIG. 3. The structural heterozygote of a new inversion over WT. This inversion was found in only one larva of Tuolumne origin. It is probably the result of spontaneous breaks in cage No. 9.

somal breaks which brought this rearrangement (now referred to as "Tuolumne") must have occurred in or in the immediate ancestry of the wild parents of this strain.

2.) A second new inversion was discovered in population cage No. 9 in the first generation count. Of 230 chromosomes examined only this one was found. It occurred in heterozygous condition with WT and was located on a WT chromosome independent of that arrangement (see fig. 3 for new inversion /WT). Since it has never been seen again in cage No. 9, the probability is greatest that this inversion arose spontaneously in cage No. 9, an event of very rare occurrence.

Jacksonville Cages

In preparing populations for cages No. 4 and No. 6 containing WT–ST and WT–MD chromosomes of Jacksonville origin respectively, it was assumed that there were no significant differences between chromosomes of Tuolumne and of Jacksonville origin. Each cage was started with high initial frequencies of WT; if the gene contents of inversions from each end of the altitudinal transect were the same, presumably the reactions of competing chromosomes would be the same in the experimental populations. On the basis of this assumption it was expected that no changes would occur at 25°C.

Cage No. 4 (WT–ST) was placed first in the 25° constant temperature room for two generations to determine the direction of trend. As can be seen from table 5, WT chromosomes *increased* very rapidly, a reaction quite different from that expected (WT increased 11.3% in two generations). It should be noted that ST/ST zygotes did not occur in the second generation sample. While it is possible that the WT/WT zygotes were equal or slightly poorer than WT/ST in adaptive value (a condition which would result in an equilibrium with WT at > 90 per cent even if ST/ST zygotes were completely eliminated in each generation) the most likely interpretation is one of

TABLE 5. *Relative frequencies of gene arrangements in cages No. 4 (above) and No. 8 (below: Jacksonville)*

Temp.	Date	% WT	% ST	Chromosomes examined
25° C.				
	Aug. 25, 1948	78.2	21.8	302
	Sept. 28	87.5	12.5	296
	Nov. 4	89.5	10.5	296
	Transferred to 16° on Nov. 17			
16° C.				
	Dec. 29	87.0	13.0	300
	Feb. 5, 1949	82.2	17.8	252
	Mar. 11	83.7	16.3	300
	Apr. 12	77.6	22.4	250

χ^2 (zygotic data at 16°) = 21.23, d.f. = 8, p. < 0.01.

Temp.	Date	% WT	% ST	Chromosomes examined
25° C.				
	Nov. 17	39.4	60.6	300
	Dec. 10	51.4	48.6	150
	Jan. 10	52.0	48.0	250
	Feb. 5	56.4	43.6	250
	Mar. 9	61.6	37.0	300

superiority of WT/WT over WT/ST. Before such a condition might create too great a deficiency of ST, the cage was transferred to 16°, and cage No. 8 containing the same strains of WT and ST was started with a low frequency of WT. It was not intended that cage No. 8 would be retained for a period long enough to allow complete elimination of ST chromosomes, but merely long enough to note the rate of change of frequency and thereby to utilize the Wright formula in calculation of the selection coefficients. After four generations at 25° WT had increased in cage No. 8 by 21.8 per cent (see table 5). It can be seen that the changes at 25° in cages No. 4 and No. 8 occur at approximately the same rates, although the rate of change in No. 8 appears slightly slower than in No. 4. A calculation of selection coefficients for the four generations of cage No. 8 gives the following results:

Genotype	W		
WT/WT	1.060	$s = -0.060$	No equilibrium,
WT/ST	1.000		eventual fixation
ST/ST	0.665	$t = 0.335$	of WT.

It may be argued, however, that the calculated adaptive values of WT/WT and WT/ST are nearly equal and that the difference is not significant. Complete elimination of ST in this cage might not take place.

After cage No. 4 had been transferred to 16° the trend suddenly changed direction and velocity. Within four generations ST had regained a foothold and had increased 11.9 per cent with an accompanying drop in WT. The trend opposite to that obtained at 25° is a real one. Selection coefficients for the zygotic classes at 16° are as follows:

Genotype	W		
WT/WT	0.909	$s = 0.091$	
WT/ST	1.000		$\hat{q} = 80.4$ per cent
ST/ST	0.634	$t = 0.366$	

It is important to note that the adaptive values of WT/WT and ST/ST dropped slightly, giving the heterozygote superiority at 16°.

Cage No. 6 (WT and MD) in many ways paralleled the changes in cage No. 4, but there were some interesting differences (see table 6). After two generations at 25°, WT had increased 23.3 per cent at the expense of MD, an apparently much faster rate than in cage No. 4, but if the changes occurring at similar frequencies are compared (78.2 per cent → 87.5 per cent = 9.3 per cent in one generation in No. 4 while 77.7 per cent → 87.6 per cent = 9.9 per cent in one generation in No. 6) the apparent overall difference in rate seems to be due to the fact that Δq is dependent on q (initial frequency) rather than to any inherent greater advantage of WT in one cage than in the other.

At the end of the second generation cage No. 6 was moved to 16° and cage No. 7 was started at 25° with only three

strains of WT and MD, all six of which were identical with those of cage No. 6. It was of interest to see whether the rate of change and direction of trend were the same for the population containing fewer strains as for the original cage No. 6 population. By referring to table 6 it is evident that the increase in WT is slower at low frequencies but becomes more rapid at higher frequencies, the total change being 24.3 per cent over a period of four generations (N.B. the final recorded change in cage No. 7 is almost the magnitude of that recorded as the first change in cage No. 6: 8.6 per cent for cage No. 7, 13.4 per cent for cage No. 6).

Calculations of the selection coefficients for cage No. 7 gave the following results:

Genotype	W		
WT/WT	1.383	$s=-0.383$	No equilibrium,
WT/MD	1.000		eventual fixation
MD/MD	0.932	$t=0.068$	of WT.

It is important to notice that in this case it is the adaptive value of homozygous MD which is closest to that of the heterozygote, whereas in cage No. 8 the adaptive value of homozygous ST was much lower than the heterozygote WT/ST. We can therefore symbolize the relative adaptive values of all five zygotic classes as follows for cages No. 7 and No. 8 at 25°:

$$\text{WT/WT} \geqq \text{WT/ST} > \text{WT/MD} > \text{MD/MD} > \text{ST/ST}.$$

(It is not possible to determine the position of MD/ST in this series from the present evidence.)

After cage No. 6 had been transferred to 16° the trend changed in velocity and direction just as in the case of No. 4, but at the end of four generations WT had dropped only 7.8 per cent, and an equilibrium seemed to be formed at approximately 80 per cent WT–20 per cent MD. This change in trend is undoubtedly real although a χ^2 calculated for the zygotic data shows that the 7.8 per cent change in four generations is barely significant. The selection coefficients calculated for the 16° cage No. 6 data gave the following results:

Genotype	W		
WT/WT	0.905	$s=0.095$	
WT/MD	1.000		$\hat{q}=80.8$ per cent
MD/MD	0.609	$t=0.391$	

These results are very interesting when compared with those from cage No. 4 at 16° (WT–ST). It will be seen that the selection coefficients of each zygotic class are very close to being equal in the two cages. However, it is most important to take note of the fact that WT and MD had definitely been at equilibrium for two generations before the selection coefficients were calculated, while there is some doubt as to the equilibrium con-

TABLE 6. *Relative frequencies of gene arrangements in Cages No. 6 (above) and No. 7 (below: Jacksonville)*

Temp.	Date	% WT	% MD	Chromosomes examined
25° C.				
	Aug. 30, 1948	64.3	35.7	246
	Oct. 3	77.7	22.3	278
	Nov. 8	87.6	12.4	306
	Transferred to 16° on Nov. 17			
16° C.				
	Dec. 29	85.0	15.0	300
	Feb. 5	80.0	20.0	300
	Mar. 11	81.3	18.7	300
	Apr. 12	79.8	20.2	258

χ^2 (zygotic data at 16°) = 14.30, d.f. = 8, p. = 0.07.

Temp.	Date	% WT	% MD	Chromosomes examined
25° C.				
	Nov. 16	36.3	63.7	300
	Dec. 12	40.7	59.3	150
	Jan. 14	45.0	55.0	284
	*Feb. 5	49.6	50.4	300
	Mar. 1	52.0	48.0	300
	*Mar. 9	57.7	42.3	300
	Apr. 7	60.6	39.4	300

* These counts represent changes within a generation since the interval from the previous count is less than a complete life cycle.

dition between WT and ST (compare results in tables 5 and 6); the possibility remains that WT and ST might actually form an equilibrium somewhat lower than that calculated on the basis of the available data (80.4 per cent $= q$).

At any rate it is possible to symbolize the relative adaptive values at 16° and take note of some interesting points:

$$WT/MD \geqq WT/ST > WT/WT > ST/ST \geqq MD/MD.$$

The apparent advance of WT/MD is especially striking when its adaptive values are compared with WT/WT at 25° and at 16°. Both heterozygotes are without any doubt superior to all homozygotes at the lower temperature. The exact position of ST/ST and MD/MD with respect to each other is somewhat in doubt owing to the near equality of their adaptive values in relation to the respective heterozygotes with WT.

According to the February collection of flies from Jacksonville (see table 1) the KL arrangement was second in frequency to WT (23.3 per cent KL, 56.4 per cent WT). The results of competition between WT and KL, therefore, are of great interest at this locality, not only for comparison with those reactions of the same arrangements from Tuolumne but also for comparison with the other arrangements at Jacksonville. Cage No. 3 (WT–KL from Jacksonville) showed no significant changes after two generations at 25° (see table 7). A homogeneity χ^2 for zygotic data was calculated: $\chi^2 = 2.89$, d.f. $=4$, p. $= .50 - .70$. Cage No. 5 was started to check on this lack of change at 25°; the initial frequencies were reversed with respect to cage No. 3 in order to show whether the lack of change was due to genuine absence of selection pressure. The results of cage No. 5 confirmed the assumption that the initial frequency was maintained according to the Hardy-Weinberg principle of equilibrium at 25°.

At 16° a slight trend was established which showed an increase of WT by 10.4 per cent in three generations. After two more generations it became evident that WT and KL had formed an equilibrium at approximately 50 per cent each. The changes are not highly significant, but it is reasonable to believe that this trend is a real one since the proportions at equilibrium remained constant for two generations.

Selection coefficients for the data in cage No. 3 at 16° are as follows:

TABLE 7. *Relative frequencies of gene arrangements in cages No. 3 (above) and No. 5 (below: Jacksonville)*

Temp.	Date	% WT	% KL	Chromosomes examined
25° C.				
	May 25, 1948			
	Initial	40.8	59.2	
	Control	37.5	62.5	296
	June 18	42.0	58.0	300
	July 22	40.0	60.0	300
	Transferred to 16° on July 28			
16° C.				
	Sept. 14	41.0	59.0	300
	Oct. 21	46.5	53.5	290
	Dec. 4	50.4	49.6	300
	Jan. 14	47.4	52.6	300
	Feb. 19	51.4	48.6	300

χ^2 (zygotic data at 25°) = 2.89, d.f. = 4, p. = 0.58.

χ^2 (zygotic data at 16°) = 18.40, d.f. = 10, p. = 0.048.

Temp.	Date	% WT	% KL	Chromosomes examined
25° C.				
	Dec. 10, 1948	72.8	27.2	250
	Jan. 6, 1949	69.0	31.0	288
	Feb. 2	71.4	28.6	300
	Transferred to 16° on Feb. 8			
16° C.				
	March 11	69.4	30.6	300
	April 13	77.2	22.8	298

χ^2 (zygotic data at 16°) = 7.48, d.f. = 4, p. = 0.11.

Genotype	W		
WT/WT	0.470	$s=0.530$	
WT/KL	1.000		$\hat{q}=51.2$ per cent
KL/KL	0.443	$t=0.557$	

The check cage (No. 5) gave slightly ambiguous results for the two generations recorded at 16°. The first generation sample showed no significant change from the last frequency at 25°, but the second sampling showed a sudden increase in WT (7.8 per cent in one generation). An explanation for this unexpected change might lie in the fact that the relative humidity for March was extremely low in comparison with other months (see appendix for relative humidity), a fact which could be a clue to the agency of selection. A χ^2 on these data gave results which show that there may be no essential reason for believing that any real change has taken place. There can be no doubt, however, that there was no net differential survival of either WT or KL at 25°. All zygotic classes were adaptively equal or nearly so. At 16° cage No. 3 clearly shows that the heteroxygote (WT/KL) is superior to both homozygotes which were in turn very nearly equal to each other; if the sudden jump of 7.8 per cent increase in WT is real in cage No. 5, then it would appear that WT/WT is clearly superior to KL/KL at 16°. It should also be pointed out that while the strains of flies were the same for both cages, cage No. 5 had two less strains of KL than cage No. 3.

We can symbolize the relations of the three classes at 16° as follows from cage No. 3:

$$\text{WT/KL} > \text{WT/WT} \geqq \text{KL/KL}.$$

It might be argued that since KL has not been used in competition with ST or MD, all the possible classes observed in nature cannot be arranged in order of superiority; but for those which are known it is helpful to combine all zygotes into their probable descending order under laboratory conditions on the basis of the calculated adaptive values (for 16°, cages No. 3, No. 4, and No. 6):

$$\text{WT/KL} > \text{WT/MD} \geqq \text{WT/ST} > \text{WT/WT} \geqq \text{KL/KL} > \text{ST/ST} \geqq \text{MD/MD}.$$

For the same cages (plus No. 7 and No. 8) at 25° the situation is quite different:

$$\text{WT/KL} = \text{WT/WT} = \text{KL/KL} \geqq \text{WT/ST} > \text{WT/MD} > \text{MD/MD} > \text{ST/ST}.$$

Discussion

Comparison with D. pseudoobscura

In studying the adaptive values of Standard, Arrowhead, and Chiricahua gene arrangements from Piñon Flats, Dobzhansky (Wright and Dobzhansky 1946) found that there were no perceptible changes in frequencies of any of the arrangements at 16° C., while at 25° ST is definitely superior to both CH and AR. Later he (1948b) compared the adaptive values of the same chromosomes from Piñon Flats, Keen Camp, and Mather, California; and in no case did any experiment show positive evidence of change in frequency at 16° (there was a slight but non-significant decrease in ST and increase in CH from Keen Camp at 16°).

These results from Dobzhansky's work on *D. pseudoobscura* contrast with the present evidence from *D. persimilis*. With the exception of cage No. 2 and the doubtful case of No. 5 all populations show some selection at 16°, and in many cases there are strong differences in adaptive values between the competing arrangements. At 25° none of the experimental populations containing Tuolumne chromosomes gave any evidence of differential survival. All the zygotic classes of WT and KL from Jacksonville are adaptively similar at 25°, while at 16° the heterozygotes are definitely superior.

These results are most interesting when the ecological preferences of these two species are kept in mind. *D. persimilis* is much more successful than its near relative under cooler, more humid conditions and at higher altitudes. The fact that the third chromosome polymorphism

has been found to be significantly adaptive at higher temperatures for *D. pseudoobscura* and at lower temperatures for *D. persimilis* is indicative of a real physiological difference between these species and gives an additional clue to the solution of the problem of differentiation which took place during the original divergence of these species.

The Question of Gene Differences in Chromosomes from Various Localities

In regard to the polymorphism of the third chromosome in *D. pseudoobscura*, it is important to know not only whether local populations differ in the frequency of arrangements but also whether the gene contents of the inversions differ between localities. The evidence seems to be overwhelmingly in favor of differences in both respects between local populations of *D. pseudoobscura* (Dobzhansky 1948b). It should be pointed out here that this fact strengthens Mayr's hypothesis (1945): "Each gene arrangement has at each given locality gene contents of specific selective value. The interference with crossing over (caused by inversions) tends to preserve the gene combinations which have local selective advantages." Consequently the problem of discontinuity in the geographic range of some arrangements boils down to "not how did these discontinuities arise but how are they *maintained?*" Dobzhansky's population cage tests on chromosomes from Piñon Flats and Keen Camp (thirteen miles apart but of quite dissimilar biotic situations) showed that ST, CH, and AR chromosomes were significantly different in their reactions, and when the results obtained from each of these localities were compared with results from Mather experiments still further differences were observed.

On the basis of such results Dobzhansky has formulated the following hypothesis of co-adaptation (1947c):

> "Individuals heterozygous for chromosomes with different gene arrangements from the same population are [with few exceptions] . . . adaptively superior to chromosomal homozygotes. Hence, the selective process leads to an equilibrium state in which all competing chromosomal types are preserved. . . . The process of natural selection, therefore, encourages the spread of those gene complexes . . . which produce superior heterozygotes. . . . This 'co-adaptation' of the gene complexes apparently takes place separately and independently in populations which reside 300 miles, or perhaps even as little as 15 miles, from each other."

The results of experimental populations of *D. persimilis* present additional evidence for genetic heterogeneity between localities. The changes in Jacksonville cages at 25° containing WT–MD and WT–ST contrast strongly with the lack of change in Tuolumne cages containing the same arrangements. At 16° the contrast is also pronounced: Jacksonsville WT–MD equilibrium = 80.8 per cent WT, $s = .095$, $t = .391$; Tuolumne WT–MD equilibrium = 57.2 per cent WT, $s = .564$, $t = .754$; Jacksonville WT–ST equilibrium = 80.4%, $s = .091$, $t = .366$; Tuolumne WT–ST, no changes in frequency, adaptive values equal. The differences between WT–KL from the two localities cannot be checked since the data from Tuolumne cage No. 9 are not extensive enough for calculation of selection coefficients.

The lack of change of inversion frequencies in any Tuolumne cage at 25° has significant implications when the temperature data from this locality are examined: (U. S. Weather Bureau Climatological Data for Ellery Lake (el. 9,600', eight miles east of Tuolumne), average temperature for July (1935–1945) = 55.0° F. (12.8° C.); highest recorded temperature for 1945 = 76° F. (24° C.), for 1946 = 71° F. (22° C.), for 1947 = 72° F.) It is obvious that flies at that elevation never experience high temperatures for any long period of time. Selection has had no opportunity to produce adaptive gene combinations under warm conditions in nature, and consequently no differences in adaptive values can be demonstrated. However, the adaptive values cease to be equal at a lower temperature

in the case of WT–MD and WT–KL; it seems likely, therefore, that the laboratory agencies of temperature, humidity, and density of population must also be at least partly instrumental in nature. The results of the cages from 25°, an exceptional temperature for Tuolumne flies, indicate that when a population which has been maintaining an adaptive polymorphism in a certain environment is placed in a situation in which selection has never had a chance act, the adaptive values of the various polymorphic types cease to be functional, and all adaptive genotypes tend to remain at a constant frequency.

It must be pointed out that in the case of WT and ST (cage No. 2 Tuolumne) no selection is demonstrable at 16°; the agency which maintains frequencies of these arrangements in nature cannot be the agency of temperature at least not in the 16°–25° range. When WT competes with MD or KL chromosomes, however, there is no question of its superiority under the 16° conditions, although not at the magnitude found in nature. Attention is called to the fact that the breeding season is very short at Tuolumne (two or four generations); consequently no appreciable seasonal cycle in frequency changes should be expected here, and selection might be especially strong during the period of hibernation. It might well be, therefore, that maintenance of inversion polymorphism is accomplished by selection at very low temperatures, considerably below those used in the present experiments.

On the other hand the environment of Jacksonville (Upper Sonoran Life Zone) supports a breeding population throughout most of the year. The strong differences in adaptive values between Jacksonville chromosomes at 25° and at 16° indicate that at least part of the adaptive mechanisms developed in nature are operating in the laboratory; however, those differences might also indicate seasonal cycles at this locality not yet demonstrated by sampling at Jacksonville. If it is assumed that the relative superiority of the arrangements in the cages parallels the situation in nature at all, the character of the adaptive polymorphism in this locality can be predicted to some extent.

At 16° there can be no doubt of the superiority of all heterozygotes with WT over the homozygotes, a fact which corroborates Dobzhansky's theory of coadaptation. The February sample from Jacksonville shows KL second in frequency to WT as shown above; and at 16° the WT/KL is superior to WT/MD and WT/ST, while KL/KL is clearly superior to ST/ST and MD/MD since it is almost equal to WT/WT at 16°. The relationships between MD and ST are less clear; their frequencies are almost equal in the February collection although MD is somewhat greater in the summer. Until competition experiments have been tried between KL, MD, and ST, no interpretation could be safely attempted with respect to the frequencies of MD and ST at Jacksonville. If the elimination of ST and MD with fixation of WT at 25° reflects the situation at warm temperature in nature, it is reasonable that there might be some advantage to the heterozygotes KL/MD and KL/ST, a possibility not yet demonstrated. This suggests that WT and KL form an efficient combination in maintaining inversion polymorphism at Jacksonville, but apparently at high elevation KL no longer forms such advantageous heterozygotes with the other arrangements since KL is very infrequent there.

At this point it can be stated that the adaptive values of the principle gene arrangements of the third chromosome of *D. persimilis* from different ecological situations contrast strongly. The genetic mechanism responsible for this contrast cannot be definitely shown from the data presented, but three possibilities become evident: (1) the gene contents of the third chromosome arrangements differ; (2) the remainder of the genotype, which may exert a modifying action on the adaptive values of the third chromosomal arrangements, may differ; (3) or, more

probably, both these possibilities may be true for each local well-adapted population. The evidence is scanty for any definite conclusion here, but some attention should be called to the following. In the Jacksonville population cages some strains of WT were used throughout, and yet the competing arrangements did not show identical behavior. At least each arrangement seems to have separate characteristics within a locality. The Tuolumne cages on the other hand had no strains in common with each other, and the reactions of the competing arrangements were much alike at 25°, but contrasting at 16°. Such contrast at 16° might be explained on the basis of modifying factors in the residual genotype, or inherent differences between the third chromosome arrangements, or both.

Additional evidence for the intralocal specificity along this transect can be found in the data for wing area and length dimensions of *D. persimilis* by Reed and Reed (1948). Such data not only show a morphological gradient in wing dimensions with altitude, but also they suggest genetic differences between populations since many of the samples are statistically different along the transect.

Attention has been called to the slight discontinuities between life zones and relative homogeneity of inversion frequencies within life zones (see fig. 1). The parallel gradient in frequencies of ST and AR chromosomes of *D. pseudoobscura* (Dobzhansky, 1948a) along the same transect gives additional evidence of homogeneity within life zones, but since the seasonal fluctuations in frequency are very pronounced in this species it is difficult to assign any particular frequencies to any one area.

Stalker and Carson (1948) have shown that the gradient in gene arrangement frequencies and morphological dimensions of *D. robusta* along an altitudinal transect in the Appalachian Mountains is somewhat bimodal in that the gradient is regular above 2,000′ and below 1,400′ but is very steep in the area between. This evidence also suggests homogeneity within life zones. In addition these authors found that two of the inversions common in northern United States showed a striking decrease in frequency with decrease in altitude, but some other clearly "northern" inversions showed no change (2R) or an increase in frequency (3R) with decreasing altitude. For this discrepancy between widely geographic gradients and smaller altitudinal gradients the authors offered two possible explanations: "(a) the environments in the north and at 4,000 feet in Tennessee are similar but by no means identical; (b) the same gene arrangement may have different genetic values at different localities." Because the evidence seems to be overwhelmingly in favor of interpopulational genetic differences for the gene arrangements of *D. pseudoobscura* and *D. persimilis,* this explanation for the discrepancies between the *D. robusta* gradients is offered here. It appears more likely that the gene contents of any particular chromosomal arrangement are determined by natural selection within each locality; if the localities are contiguous, migration between them will tend to equalize their genetic differences and will result in a gradient between those localities. However, the same arrangement from two distant localities of diverse ecological patterns or of the same pattern but separated by another sort of environment may possess very different gene combinations. The mechanism of gene arrangement polymorphism then is useful to the species by the different gene combinations which can be acquired to fit the local population to its environment. Presumably certain alleles may be retained in one locality and other alleles of the same loci at other localities. These alleles would be selected on the basis of their utility to the heterozygote ("co-adaptation") but would not be as useful to the cross between localities of different ecological pattern; therefore, even though migration would take place between localities, the gradient would be preserved as long as the ecologi-

cal patterns remained different for each local population. Although these considerations by no means solve the vast problem of gradients, they do throw some light on the sort of pattern to be reckoned with in its solution.

Summary

Four well-established gene arrangements of the third chromosome of *Drosophila persimilis* form a gradient in relative frequency along an east-west transect on the western slope of the Sierra Nevada. Populations of flies with predetermined relative frequencies of these chromosomes were maintained for several generations under controlled conditions for the purpose of investigating the adaptive values of various combinations of the gene arrangements. The data obtained from nature and from the experimental populations show the following:

(1) The adaptive values of gene arrangements from the high elevations (Tuolumne, California) are equal at 25° C. All gene arrangements from low elevation (Jacksonville) however, are not equal at 25°: homozygous Whitney is equal or superior to heterozygotes with Standard and Mendocino, and the tendency is for elimination of the two latter with consequent fixation of WT. Whitney and Klamath chromosomes from low elevation appear to be nearly equal at 25°.

(2) At 16° high elevation Whitney chromosomes are superior to MD and KL but equal to ST. At low elevation WT is superior to all three, but equilibria are formed in all cases at 16°, a fact which indicates superiority of heterozygotes over homozygotes. This fact corroborates Dobzhansky's theory of co-adaptation of gene contents within chromosomes from the same population.

(3) The lack of adaptive differences between chromosomes from high elevation at 25° probably indicates that the agency of selection in nature is not being reproduced in the laboratory. The fact that selective changes in frequency do occur to some extent but not at the magnitude observed in natural populations when these arrangements compete at 16° indicates that the temperature difference, humidity, or density of population, or any combination of these, may be partially instrumental in nature.

(4) A comparison of results of these population experiments indicates considerable difference in adaptive value and quite probably therefore in gene contents of the same gene arrangement (WT, ST, and MD) from the localities at opposite ends of the altitudinal transect. The present evidence does not indicate a significant difference between Tuolumne KL and Jacksonville KL.

(5) These results also indicate that the third chromosome inversions in *D. persimilis* are more adaptive at lower temperatures, a fact which is in strong contrast with the situation in *D. pseudoobscura* whose chromosomal variants are more different with respect to adaptive values at higher temperatures. This contrast may be indicative of a real physiological difference between these species.

(6) The evidence from *D. pseudoobscura, D. persimilis,* and *D. robusta* populations along various altitudinal transects has pointed to homogeneity of inversion gene contents within life zones and slight heterogeneity between life zones. Evidence from morphological dimensions of these species also indicate that many populations are significantly distinct.

(7) Some general considerations to be dealt with in solving the problem of altitudinal gradients are summarized.

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APPENDIX A

Relative humidity data (mean monthly average)

Temp.	Per cent humidity	Month	Number readings
16°	83	March, 1948	1
16°	83	April	2
16°	89	May	1
16°	83–89	June	4
16°	83.5±0.9	July	20
16°	83.0±0.6	August	20
16°	82.5±0.6	September	23
16°	72.2±1.0	October	23
16°	72.9±0.9	November	17
16°	69.1±0.7	December	22
16°	75.1±0.7	January, 1949	21
16°	67.0±1.0	February	22
16°	62.7±0.8	March	26
25°	70–74	June, 1948	4
25°	73.6±0.9	September	21
25°	67.2±0.7	October	26
25°	68.8±1.0	November	23
25°	66.6±0.8	December	25
25°	65.9±0.4	January, 1949	24
25°	73.5±1.3	February	22
25°	71.7−0.8	March	25

APPENDIX B

1947 Climatological Data (U. S. Weather Bureau)
Normal average temperature for past ten years

Month	Sonora (el. 1825′) (9 mi. N. of Jacksonville)	Ellery Lake (el. 9600′) (8 mi. E. of Tuolumne)
J	44.5°	22.1°
F	47.9	23.0
M	51.6	27.2
A	57.0	31.6
M	63.1	38.2
J	70.7	46.0
J	77.9	55.0
A	76.3	54.3
S	70.1	47.7
O	60.7	39.5
N	51.9	31.5
D	45.8	25.9
Normal Annual average	59.8°	36.8°

Normal average precipitation for same period

	Sonora	Ellery Lake
J	6.39″	4.93″
F	6.10	5.46
M	5.80	3.75
A	2.59	2.81
M	1.43	1.31
J	0.31	1.03
J	0.01	0.62
A	0.02	0.71
S	0.41	0.49
O	1.73	1.43
N	3.08	2.32
D	5.30	4.49
Normal Annual total	33.17″	29.90″

NATURAL SELECTION IN LABORATORY POPULATIONS OF *DROSOPHILA*.[1] II. COMPETITION BETWEEN A WHITE-EYE GENE AND ITS WILD TYPE ALLELE

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Introduction

Reed and Reed (1948) described a useful technique for studying competitions between different strains of Drosophila in the laboratory. The "Population bottles" in which the competitions may continue to their natural ends, are easy to assemble and maintain. Their effectiveness was demonstrated by a study of the selective advantage possessed by an inversion heterozygote over the two related homozygotes in *Drosophila melanogaster,* and reported in the paper cited above.

The data to be presented here include the results of a competition between a sex-linked gene causing white eyes and its wild type allele in *Drosophila melanogaster.* The gene for white eyes is recessive to its wild type allele which causes red pigmentation of the eyes. Inheritance of a sex-linked gene such as that for white eyes is distinctive in that males have only a single member of a pair of alleles present instead of two members. Consequently, if the white gene is present in a male it always shows but in females if only one white gene is present it is "covered-up" by the red wild type allele and does not show. The gene for white eyes was eventually eliminated from the competition and it is clear that its effect was *not* primarily concerned with the *viability* of white versus wild type flies. The disadvantage of the white allele at eclosion compared with the wild type (red) allele seems to be mainly a result of the relatively greater success of wild type males in securing matings with both red and white females.

There can be no doubt that the competition between males of different genotypes for mates is a very important factor in natural selection and it is reasonable to expect that there will be an extensive increase in the information available in this field before long.

Materials and Methods

The reader is referred to the paper of Reed and Reed (1948) for a description of the population bottle technique. Except where indicated otherwise all stocks and experiments were in a constant temperature room held at 20° C. ± 0.5° C.

All the flies were descendants without outcrossing from a somewhat "isogenic" stock which had been derived in the following fashion.

Generation 1. ♂ white × ♀ wild (Lausanne Special).

Generation 2. ♀ heterozygote (from 1) × ♂ wild (Lausanne Special).

Generation 3. ♂ white (from 2) × ♀ wild (Lausanne Special).

Generation 4. ♀ heterozygote (from 3) × ♂ wild (Lausanne Special).

This process was continued for thirty generations. Each generation in both the pure Lausanne Special stock and its derivative with the white gene present was produced by a single pair of flies. Thus the white gene, along with a few closely adjacent genes, was introduced into the inbred wild type Lausanne Special strain. An approximately isogenic stock should be a minimum prerequisite for any study of a competition between two alleles. The use of a sex-linked gene for the study has the advantage that spontaneous attempts of the stock to set up a balanced lethal

[1] Research accomplished at Harvard University and supported by a grant from the Milton Fund.

system would be detected at once, due to the deficiency of males, and prevented. The greatest advantage in the use of a sex-linked gene such as white comes from the fact that in studies of sexual preferences one can tell whether or not a particular female has mated with *both* the competing types of males or with only one type by observing her offspring. That is, one can tell which type of male she mated with and recognize the double-mating.

Viability of White and Wild Type Flies

The relative viability of the flies of the competing genotypes from the time the egg is fertilized until release from the pupa case has occurred can be determined easily enough by counting the flies as they emerge. This was accomplished and two treatments, temperature and crowding, were varied sufficiently to indicate whether they might be important causes of the results to be observed later in the competition. In these experiments F_1 females heterozygous for white were always mated with white males. This single kind of backcross eliminates any competition between parents with different genotypes as the mothers all have one genotype (Ww) and the fathers all are alike (wY). The progeny from this cross are expected to segregate in equal numbers of red and of white animals, if there has been no differential survival of white flies compared with red.

Four experiments were carried out utilizing the four possible combinations of controlled temperature at 20° C. and room temperature at about 24° C. with lack of crowding (5 pairs of parents) and some crowding (25 pairs of parents). There were no significant deviations from the expectation of equal numbers of red and white-eyed flies in the four experiments. The grand total consisted of 12,979 flies with the wild type red phenotype (6,032 males and 6,947 females) to 12,927 white flies (6,083 males and 6,844 females).

The evidence is clear that neither the white-eyed nor red-eyed phenotype shows any selective advantage in viability from fertilization of the egg until appearance of the adult. Nor did moderate modifications of temperature and crowding of parents have any effect upon the survival of the different phenotypes of the offspring.

The evidence is likewise clear that males do not compare favorably with females in viability. Of the total of 25,906 flies in the four experiments there were 12,115 (46.8%) males to 13,791 (53.2%) females. The deviation from equality is 10.4 times its standard deviation. The failure of males to show equal viability to that of females has been observed by many authors and it is in no way associated with the white-eye gene locus.

Selective Mating of the Red Versus White Phenotypes

A number of interesting papers have been published by Dobzhansky and others which show that some matings between the same or different strains or species of Drosophila have a significantly higher frequency than others. Perhaps the most frequent type of experiment has been that in which males of one strain were placed with females of two strains, one of which was the same as the strain of the males. It could be assumed that the males "selected" one of the two types of females more frequently than the other. However, it is more likely that the observations are more a measure of the ability, or lack of it, of the alien females to repel the males which fail to stimulate them to the point of copulation.

Bateman (1948) states that, "It would seem desirable to carry out matings in which females were given a choice of males. Though there would be great technical difficulties, it would be informative to obtain comparative data from both types of mating." Perhaps, however, the technical difficulties are more apparent than real. If it cannot be told from the progeny what type mating has occurred then different types of flies may be marked

by small nicks in the wings and the matings observed directly. This takes time but there are usually friends, relatives or students who are willing to serve as volunteer fly watchers. The use of sex-linked mutants to demonstrate the principles of selective mating is not at all difficult as demonstrated by Spett (1931) and Diederich (1941). Modifications of their techniques have been used in our work with the white-eye gene.

Very small bottles, "creamers," were made up each containing four virgin flies, less than eight hours of age. The four flies had these genotypes, ♀ Ww, ♀ ww, ♂ WY, and ♂ wY and remained together for 24 hours after which the males were discarded and each female was placed in a fresh creamer and left there by herself until the pupae, if any, appeared. At this time the female was discarded, a record having been kept of her eye color.

If the female had white eyes and all her daughters had white eyes then the white male had succeeded in mating with her. If all the daughters had red eyes then the wild-type male was the father. If there are both red and white eyed daughters in any ratio, then at least one male of both competing genotypes had succeeded in mating with the white mother, such instances being called "double matings."

If the female had red eyes and all her daughters have red eyes the father was the red-eyed male. If half of the daughters had red and half white eyes the father was the white eyed male. Any other true ratio of red to white-eyed daughters is a result of double matings. The number of double matings here is open to some small error but the number expected can be predicted from the results of the white-eyed mothers which are not subject to this particular error. It is easy to make this error negligible by keeping the number of double matings at a very low frequency. This is done by not allowing the competing males to be with the females for more than 24 hours. This precaution increases the routine labor considerably because 234 (39 per cent) of the 603 females which survived in isolation for more than one week produced no offspring and presumably were in most cases virgins. Females which died before they had been isolated for one week are not included in the data which accounts for the fact that the number of white and red females is not identical as shown in table 1, which gives the results of this experiment.

TABLE 1. *Results of the selective matings when both red and white phenotypes of both males and females are present*

	Mating of white ♀	Mating of red ♀	Totals
White ♂	90	64	154
Red ♂	100	105	205
Double mating	6	4	10
No offspring	116	118	234
Totals	312	291	603

The ten double matings shown in the table mean that ten red and ten white males were successful in mating with the same ten females. If ten males are added to the proper totals there will be 215 red males which succeeded in copulating, to 164 white males, a rough ratio of 1.00 to 0.75. The deviation from equality is statistically significant but not highly significant.

Probably the low frequency of double matings is a result of the tendency of females which have mated to repel a second male for some time. Rendel (1945) observed that females of *Drosophila subobscura* repel courting males immediately after mating. Mayr (1946) observed that females of *D. pseudoobscura* and *D. persimilis* were non-receptive for at least one hour after copulation.

The data of table 1 indicate that white males were discriminated against by both white and red females and especially so by the red females. Red females mated with white males in only 38 per cent of the cases but white females mated with white males 48 per cent of the time. We

have no information as to why red males are accepted for copulation more frequently than white males. Perhaps there are many different factors involved. Whatever the factors concerned the differential acceptance of the two types of males over a span of many generations would decrease and probably eliminate the gene for white eyes from the population. The following experiment was done to test whether the rate of elimination of the white gene is roughly what would be expected from this mating differential.

Competition to Extinction

Twenty population bottles were started on April 2, 1946. Due to one accident or another, such as contamination by molds or other Drosophila, breakage of bottles, or escape of a significant number of flies during change of food bottles, the twenty lines gradually dwindled to three and the experiment was finally terminated on March 26, 1948. By then the white gene had become extinct, as evidenced by the failure of any white males to appear during the last seven months of this period (about ten generations). The population bottles were kept in a constant temperature room at 20° C. during the two year period except for occasional short periods of time when they were removed for demonstration purposes.

Each bottle was started with a total of twenty unmated flies, five red males, five white males, five white females and five heterozygous red females. This system has the advantage of an equal frequency of all four phenotypes at the beginning of the experiment. Hindsight shows, however, that the experiment will proceed faster if the white females are not included in the initiation of the experiment. This latter system was adopted in studies of yellow, forked, raspberry and cut to be reported on by Merrell and Ludwin at a later date.

Returning to the system actually used with the white gene, as stated above, there will be three times as many white genes present as wild type in the mothers. The males are equal as to red or white. The total gene frequency in the parents will be two white to one red.

In calculating the expectations for red and white flies of the next generation we must take account of the observed fact found in the previous section on selective mating that the ratio of successes is 1.0 for red males to 0.75 for white males. Consequently the expectation of genotypes in the first generation of daughters in the population bottles will be found as shown below. The sons should be in the ratio of 3 white to 1 red.

		Mothers' gametes	
		1 W	3 w
Fathers' gametes (ratio of success)	1 W	1 WW	3 Ww
	0.75 w	0.75 Ww	2.25 ww

From the above the percentage of daughters and sons of the various genotypes to be expected can be calculated with the following results:

Daughters	7.2% WW	26.7% Ww	16.1% ww
Sons	12.5% WY		37.5% wY

Before we combine phenotypes, however, one further weighting should be included, that of the deficiency of males in relation to females which is independent of eye color. In the section on viability it was stated that for all four experiments there were 12,115 (46.8 per cent) males to 13,791 (53.2 per cent) females. With these figures we may compare the counts of males and females obtained during the competition to extinction of the white gene in the population bottles. There were only 4,680 (43.3 per cent) males to 6,122 (56.7 per cent) females in the counts made of the flies in the population bottles. Apparently conditions of crowding, etc., were more severe in the population bottles than in the viability experiments, as the deficiency of males is greater in the population bottles. Consequently

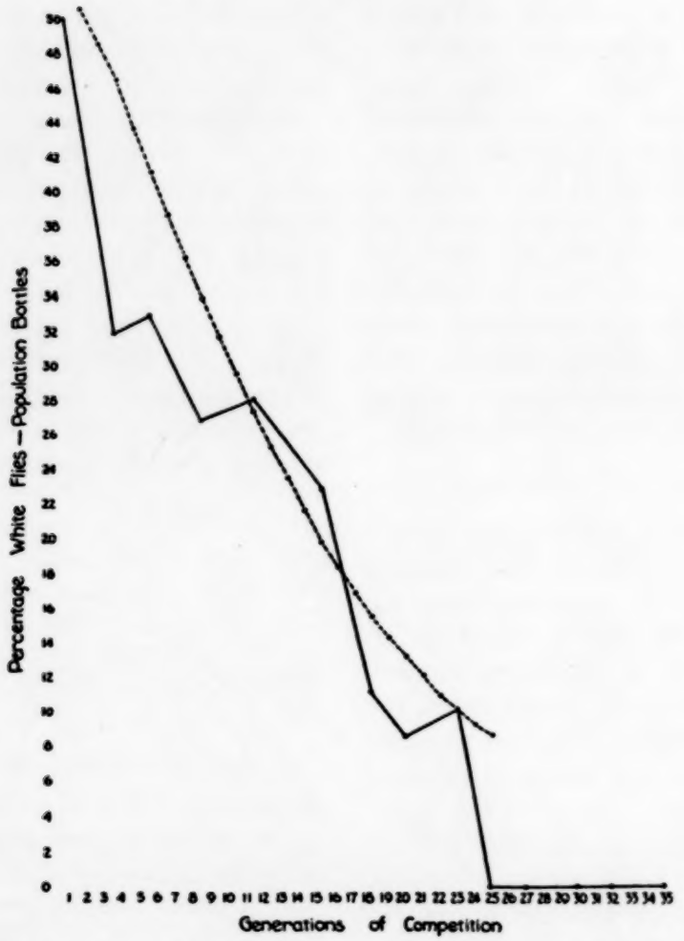

FIG. 1. Competition to extinction. *Solid* line is the observed curve for the loss and extinction of the white gene from the population. The loss is shown by the phenotypes of males and females combined. The *broken* line is the curve calculated for combined male and female phenotypes with white males at a disadvantage in selective mating as described in the text.

the proportions of females which will be found in the population bottles will be as 57 is to 43 for males. Multiplying the expected values for the different genotypes of females and males by 0.57 or 0.43 gives the new expected results as follows:

Daughters	8.2% WW	30.4% Ww	18.3% ww
Sons	10.8% WY		32.3% wY

Without regard to sex the above gives 49.4 per cent flies expected to have red eye color and 50.6 per cent white eye color. This is the first point on the dotted curve of figure 1 which gives the expectation for white and red flies with the selective mating ratio for eye color of 1.00: 0.75 and the viability difference of females over males of 0.57 : 0.43 also included.

In the next generation the sons will be in the ratio of their mothers' gametes, or 0.468 W to 0.67 w. The proportions of daughters are given in the body of the diagram at top of next page.

After weighing these proportions for the differential viability of the sexes we get an expected value of 51.4 per cent red-eyed flies to 48.6 per cent white-eyed. This gives the second point on the theoretical curve of figure 1. These calculations were continued until the expectations for the white and red-eyed flies had been obtained, and the curve plotted, for

		Mothers' gametes	
		0.468 W	0.67 w
Fathers' gametes	.108 W	0.051 WW	0.072 Ww
	0.242 w (0.75×0.323)	0.113 Ww	

25 generations. The expected value for each genotype, expressed as a percentage, is given in table 2. The observed values for white and red flies of each sex are given in table 3. The material of both tables appears in figure 1.

TABLE 2. *Expected percentages of the competing genotypes. Calculated on the assumption that decrease of the gene for white-eyes is due to the selective mating ratio of 1:00 red to 0.75 white and differential survival of females to males of 0.57 and 0.43. Genotypes 1. and 2. will have the same phenotype*

Generation	Genotype				
	1. ♀ WW	2. ♀ Ww	3. ♀ ww	4. ♂ WY	5. ♂ wY
0	0	25.0	25.0	25.0	25.0
1	8.2	30.4	18.3	10.8	32.3
2	7.3	26.5	23.2	17.6	25.4
3	10.0	27.9	19.1	15.5	27.5
4	10.3	27.7	18.8	18.2	25.0
5	11.8	28.5	16.6	18.3	24.7
8	15.8	28.5	12.7	21.7	21.3
11	20.4	27.4	9.2	24.7	18.3
15	26.7	24.8	5.5	28.6	14.4
18	31.4	21.8	3.8	31.2	11.8
20	34.2	19.8	3.0	32.7	10.3
23	38.4	16.8	1.8	34.6	8.4
25	40.7	15.0	1.3	35.7	7.3

The curve calculated on the basis of the performance of the red versus white males (with the white males only three-quarters as successful as the red) is enlightening as it demonstrated how fast the less successful gene is lost due to this one factor alone. The fact that the agreement between the calculated and observed curves is not precise is entirely acceptable as the agreement is sufficiently good to demonstrate that selective mating is the most important factor concerned. If there are other factors concerned they cannot be of great importance, or if they are, they cancel out, as the two curves are in fundamental agreement.

Probably the major reason for the lack of smoothness and symmetry of the observed curve is the random gene fluctuation resulting from the small population size in individual bottles. The total population size became small enough toward the end of the experiment so that the white gene was suddenly and completely eliminated. Had the population size been continuously larger the elimination would have been slower and more gradual.

TABLE 3. *Observed percentages of the competing phenotypes. Generation numbers calculated on a basis of 21 days per generation*

Generation	Number of bottles	Phenotype			
		1. Red ♀ ♀ (WW and Ww)	2. White ♀ ♀ (ww)	3. Red ♂ ♂ (WY)	4. White ♂ ♂ (wY)
0	20	25.0	25.0	25.0	25.0
3	20	45.9	14.0	21.7	18.4
5	15	39.7	11.9	27.0	21.4
8	12	47.1	9.5	26.0	17.4
11	11	45.2	8.6	26.6	19.6
15	8	47.8	7.4	29.4	15.5
18	4	59.9	0.0	29.0	11.2
20	4	55.5	0.2	35.6	8.4
23	3	56.6	3.0	33.2	7.2
25–35	3	No white flies of either sex were found			

Professor Sewall Wright most kindly read this paper before it went to press. We wish to express our deep gratitude for his careful criticism and for the following suggestions which he contributed.

He suggested that in addition to curves showing the observed and expected phenotypes during competition, the reader would like curves for the sexes separately. Professor Wright, therefore, drew the necessary four curves after converting the data in tables 2 and 3. His rough graph, redrawn, is given as figure 2.

His other important suggestion was that we should also include a table giving the frequency of the white gene in the population for each generation and that the value be obtained as $\bar{q}$ from the expression $\bar{q} = [(2/3)\, q_f + (1/3)\, q_m]$. The value of q_m is given directly as the proportion of whites among the males. The square root of the proportion of whites among the females gives an approximation to the true value of q_f.

Table 4 gives the frequency of the white gene calculated by the formula above for the observed flies and for the calculated values assuming, as before, a selective advantage of red to white males of 1.00 to 0.75. The writers have made the calculations of table 4 and have plotted the two curves which may be obtained from the data. The curves were very similar

TABLE 4. *The drop in frequency of the white gene in the observed population and in the calculated population on the assumption of a selective advantage of the red to white males of 1.00 to 0.75. Values for both the observed and calculated populations obtained from the formula,* $\bar{q} = [(2/3)q_f + (1/3)q_m]$

Generation	$\bar{q}$ for observed populations	$\bar{q}$ for calculated population
0	0.667	0.667
3	0.473	0.602
5	0.468	0.550
8	0.403	0.463
11	0.407	0.411
15	0.363	0.321
18	0.093	0.263
20	0.111	0.229
23	0.197	0.179
25	0.000	0.150

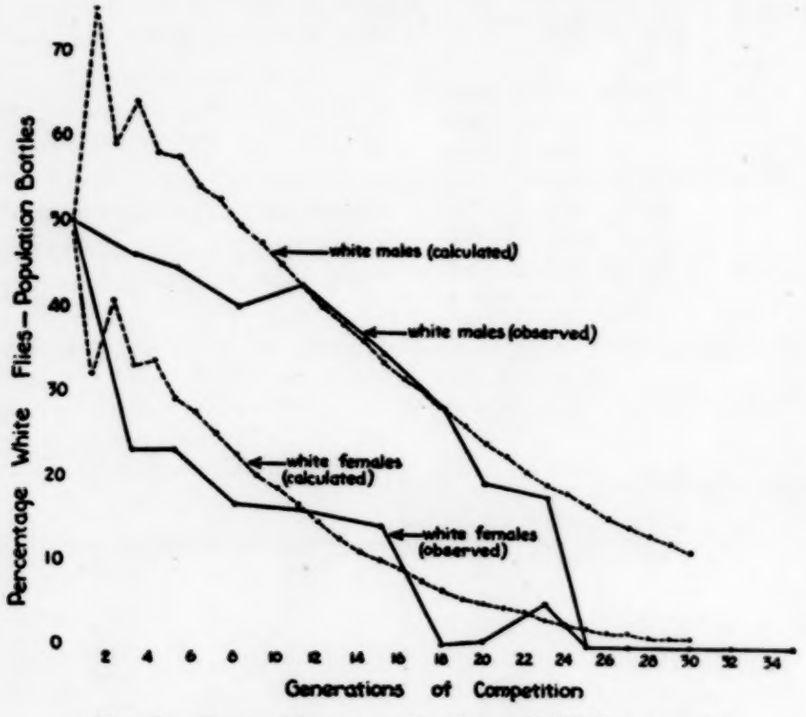

FIG. 2. Competition to extinction. *Solid* lines are the observed values for white males and white females, the *broken* lines the curves calculated for males and females expected with white males at a disadvantage in selective mating as described in the text.

in shape to the two shown in figure 1. This is not surprising as one would expect the observed phenotypes and the calculated gene frequencies to reflect each other. Because of this similarity, and in the interests of printing economy, the curves on gene frequency will not be included. They can be plotted easily from table 4 if the reader is interested.

Discussion

Genes with lethal or grossly deleterious effects obviously act directly to eliminate the organism possessing them from the population. In case of a mildly deleterious gene such as that for white eyes one can assume that an individual with white eyes is at a disadvantage in competition with one having the wild type phenotype. It is of interest to know whether such mildly deleterious genes exercise their damage via selective mating or as a direct result of the diminution of physical fitness as evidenced by survival of the fly, egg production or similar factors. No doubt physical fitness, in the broad sense, is the important reason for selection in mating. But the importance of selective mating as the evolutionary mechanism by which the mildly deleterious gene may be eliminated may have been underestimated in the past.

Wallace (1948) studied the behavior of the chromosome carrying the "sex-ratio" condition in *Drosophila pseudoobscura* in laboratory competitions. All sorts of factors are involved in this situation, as one would expect when working with a condition involving a whole chromosome with three inversions present. The factors evaluated for adaptive value can be ascribed to the sex ratio chromosome but not to individual genes upon it.

Da Cunha (1949) worked with a gene affecting the color of the abdomen of *D. polymorpha* which, under laboratory conditions, has increased survival of the heterozygous type. As the author points out, "Attempts to clarify the mechanism of action of the selective process at work have not met with success!" It was indeed a difficult problem as there were no isogenic strains of that species available. Considerable progress was made even though the effects noted could not be attributed entirely to the color genes.

Bateman (1948 and 1949) provided information on selective mating for various mutations and it would be most interesting if someone would test these values in extinction experiments in some form of population cage.

Merrell (1949) found the selective mating values for the sex-linked genes yellow, raspberry, cut and forked in stocks approximately isogenic with the Lausanne Special stock used in the experiments of the present paper. Ludwin and Merrell are now carrying out the extinction experiments in population bottles for the above four genes.

It is reasonable to assume that studies with a single allelic difference, or as close to it as "isogenic" strains can come, would have few non-canceling variables other than those resulting from the main gene difference. The agreement between the results observed and those expected for the white gene in competition, would indicate that its effect on the animal is via selective mating mainly and only secondarily through other factors which might cause elimination of white-eyed flies because of decreased physical fitness. Were there other important factors than selective mating which caused elimination of the gene, the frequency of white flies observed should have dropped below the calculated curve at a continuously accelerating rate. In order to prevent this the white flies would have to have characteristics superior to the wild type competitors. This is not a likely assumption and the observed curve did not drop away from the expected until the white gene itself disappeared. Consequently, selective mating seems to be the most important mechanism by which the white gene is eliminated from laboratory competitions.

Conclusions

The sex-linked gene which is responsible for white eyes in *Drosophila melanogaster* is a good example of a mildly deleterious character which mutates with some frequency in nature but fails to establish itself in an appreciable part of the population. The mating behavior and viability of white-eyed flies and of the wild type red-eyed flies were studied under laboratory conditions, and with approximately isogenic stocks.

It was found that the flies with white eyes and those with red eyes were equally viable when tested at two different temperatures and at two conditions of crowding.

The mating behavior of white and of red flies was strikingly different. Both red and white females discriminated against white males. The ratio of red males which succeeded in mating, compared with white males was 1.00 to 0.75. Using this ratio, a curve was calculated for the expected decrease in percentage of white flies from the fifty per cent white at the beginning of a competition until the white gene was actually found to have become extinct. The curve for the observed decrease in white-eyed flies agrees roughly with the curve calculated for the selective mating ratio of 1.00 red males to 0.75 white males. If there were other important factors concerned with the elimination of the white-eyed flies from this "isogenic" strain the observed curve would have to drop away from the calculated curve at an accelerated rate. As the observed curve shows no such phenomenon, the most important factor contributing to the decrease of the white gene in this laboratory competition, is apparently that of selective mating.

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THE ECOCLIMATIC DIVERGENCE OF *ANOPHELES BELLATOR* AND *A. HOMUNCULUS*[1, 2]

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INTRODUCTION

A well defined ecological complex stands behind the endemic malaria of central Trinidad B.W.I. Transmission is due to two closely-related forest anophelines, *Anopheles* (*Kerteszia*) *bellator* D. and K. and *A.* (*K.*) *homunculus* Komp.[4] Both these species breed exclusively in the interfoliar niches of water found in bromeliads. Malaria occurs where the peculiar climatic requirements of both the bromeliad flora and the mosquitoes are met in cacao plantations immediately abutting villages (Downs and Pittendrigh, 1946 and 1949).

The ecology of the bromeliads in general, and in their peculiar role as anopheline breeding ground, has already been discussed elsewhere (Pittendrigh, 1948, 1950a). The two remaining papers of this series are concerned with the ecological relationships of the two anopheline species. An appendix to the present paper discusses the ecology of the mosquitoes in relation to their status as malaria vectors in various parts of the island of Trinidad.

The ecological study of the mosquitoes has been directed at answering the following questions.

(1) To what extent are the distributions of the two species (which are for the most part sympatric in Trinidad) different?

(2) Are the differences that do exist determinate in the sense that they can be related to an adaptive differential between the species?

(3) What are the ecological relations between the species in the area where they co-exist? Are they out of competition with respect to each other as theoretically is to be expected? (cf. Crombie, 1947).

(4) How are adaptive differences responsible for geographic replacement related to the adaptive differences eliminating or reducing competition in the overlap area?

(5) What bearing do the ecological relations between the species have on the problem of their origin?

Two kinds of interspecific ecological differences have been detected: (1) a difference in microclimatic requirements and (2) a difference in the utilization of different bromeliads as breeding places.

The present report is concerned purely with an analysis of the microclimatic differences between the species as they relate to the first two questions above: i.e., to distribution differences caused by an adaptive differential between the species.

The ecological problems of the coexistence of the two mosquitoes in the

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[1] This paper is No. III in the series "The Bromeliad-Anopheles-Malaria Complex in Trinidad, B.W.I."

[2] The studies on which this paper is based were made under the joint auspices of the Trinidad Government and the International Health Division of the Rockefeller Foundation.

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[4] The adult females of these two species are almost identical: the only usable distinction is the relative size of the white bands on the fifth tarsal segment of the hind legs. The males are easily separated by the same character as well as by their genitalia (Komp, 1937). The larvae usually differ in color and the postspiracular hair but neither of these characters were sufficiently reliable in our experience to be used for certain identification. All larval samples were therefore reared for identification. *Anopheles* (*K.*) *anoplus* and *A.* (*K.*) sp. nov., cited by Downs, Gillette, and Shannon (1943) are known only from single specimens and there is reason to doubt the validity of both species. *A.* (*K.*) *bellator* and *A.* (*K.*) *homunculus* are, then, the only bromeliad breeding anophelines in Trinidad.

large overlap area, and the general evolutionary significance of all the results, are reserved for the following paper.

Since a variety of different problems and techniques is involved methods are described *in passim*. The general climate of the island and the structure of the vegetation types involved in this work were described previously (Pittendrigh 1948).

The Geographic Distribution of the Two Anopheline Species

Methods. The geographic distribution of the anophelines is intimately related to the distribution of rainfall in Trinidad. In this discussion reference is made constantly to seven climatic zones which are delimited primarily on the basis of rainfall. These zones, indicated in figure 1, are designated A through G, and have been described in a previous paper of this series (Pittendrigh, 1948) to which the reader is referred for a detailed map of the distribution of rainfall. A is the wettest zone with over 250″ rain per annum; B has about 200″; C, which comprises two distinct areas, has about 125″; D has 90″–110″; E, 70″–90″; F, 50″–70″; and G has less than 50″.

Data bearing on the variation of mosquito densities in the various rainfall zones are given in table 1 and figure 1. The adult densities are derived from 118 man hours of catching (human bait) during the peak period of mosquito activity from 5:30 P.M. to 7:30 P.M. All these captures were made in the wet season near habitations in cacao plantations.

The procedure employed in evaluating larval densities in the various zones requires explanation. The sampling in each zone was made by first recovering the total larval population from 60 plants of *Gravisia aquilega* which is the largest bromeliad regularly encountered in cacao plantations throughout the island. The 60 plants in each sample were all taken from branches below 20 feet above ground because the density of larvae is known to fall sharply at higher levels (Pittendrigh, 1950a). The mean density of larvae per plant of *Gravisia aquilega* is in itself not an accurate estimate of larval density for the given rainfall zone, since the densities of all the bromeliad species

Table 1. *Geographic variation of the anopheline population in rainfall zones*

		Rainfall zones				
		B Ca. 200″	C Ca. 125″	D 90–110″	E 70–90″	F 50–70″
A. Adult mosquito captures 5:30 P.M.–7:30 P.M. Wet seasons 1942–44						
(1) No. of samples (total man-hours in brackets)		5(8)	7(38)	21(37)	6(20)	11(15)
(2) Mean densities in mosquitoes/man-hour	b	1.0±0.7	24.9 ±1.6	30.1 ±11.8	3.2 ±1.8	0.2 ±0.2
	h	12.4±4.0	4.2 ±1.4	0.1 ± 0.1	0.1 ±0.1	0.0
(3) h/b		12.4	0.2			
B. Larval densities, July 1945						
(1) Breeding ground units† per tree			23.5	36.1	29.1	11.1
(2) Larval density per plant of *G. aquilega*‡	b		1.41± .3	0.60± .1	0.24± .1	0.03± .03
	h		0.40± .4	0.00	0.00	0.00
(3) Estimated larval density per tree	b		33.1	21.7	6.9	0.3
	h		9.4	0.0	0.0	0.0
C. Miscellaneous Larval Samples						
(1) Total bromeliads examined (all spp.)		1194	359	1409	187	193
(2) Total larvae		431	1058	1755	52	0
(3) h/b		27.8	0.02	<0.01	0	0

† Each unit equivalent to 1 plant of *G. aquilega*.
‡ Mean density based on a sample from 60 plants.
b = *bellator*.
h = *homunculus*.

are subject to variation, and independently of each other, from zone to zone (Pittendrigh, 1948). This difficulty was met as follows. A survey was made of the bromeliad flora [5] in each zone concurrently with the larval sampling. It yielded data on the mean frequency per tree of all bromeliad species. A coefficient, termed the "breeding index," has been experimentally obtained for all the bromeliad species involved (Pittendrigh, 1950a). It expresses as a decimal fraction the number of larvae a given bromeliad species supports relative to the number supported by *Gravisia aquilega* under comparable experimental conditions. Clearly, if the frequency per plantation tree of all bromeliad species is multiplied by the appropriate breeding index, and all the products summed, we arrive at an estimate of the size of the total breeding ground per tree. This is itself an estimate of the size of the breeding ground per unit area because plantation trees are spaced constantly throughout the island. It is to be noted that the size of the breeding ground is expressed in units each equivalent to one plant of *Gravisia aquilega.* Since the density of larvae per plant of *G. aquilega* has been determined by direct observation we can then estimate larvae per unit area.

Distributions. The two anophelines have a fairly wide total distribution in Trinidad which is centered in the wet northeast part of the island. One of the most striking features of the distribution is the very great area in which the two species coexist (fig. 1). There is a relatively small region in the wet mountain valleys in the northeast (zone B) where the population is composed almost completely of *homunculus.*[6] However this area is certainly not more than 10 per cent of the total *homunculus* range which is therefore virtually included in that of *bellator.* It is in zone B that *homunculus* densities are at their maximum; and this is the wettest section of the island where either species occurs. The incidence of *homunculus* declines sharply in the lower reaches of the valleys (zone C) and continues declining to the south and west as rainfall level decreases. Zone D is probably the driest area in which this species ever occurs, even in forest. Here it is already absent from cacao plantations. (There are marked differences between the mosquito populations of forest and plantation in each zone, and the significance of this is explained later.)

A. bellator populations on the other hand are not at their maximum density in the wettest areas. This species becomes progressively more abundant from zone B through zone D: it then becomes less abundant again in the drier E and F zones where *homunculus* is already entirely missing. *A. bellator* is itself completely absent from areas with 50″ rain and less (zone G) even though, as will be further discussed later, adequate breeding facilities in the form of water holding bromeliads are present.

The general correlation of mosquito density with rainfall level is clear, and further analysis is undertaken on the working hypothesis that the distributions are to be accounted for in terms of an interspecific difference in water relations. Thus it is supposed that *homunculus* tolerates and requires higher humidity levels than *bellator.*

Clearly if there is such an adaptive differential between the species it should manifest itself in their microdistribution on humidity gradients in the area of overlap where the species can be compared. The hypothesis is tested in this way. The distributions of the two mosquitoes are compared as follows: (1) on the profile of forest which offers a well marked gradient of microclimate, wettest near the ground and driest in the canopy (cf. Pittendrigh, 1948): (2) in the daily cycle of feeding activity which is associated with a pronounced cycle of microclimatic change: (3) in adjacent vegetation types which by virtue of different heights and densities offer different internal microclimates.

[5] The results of this survey of the geographic variation of the bromeliad flora have already been discussed in some detail from the botanical viewpoint (Pittendrigh, 1948).

[6] In addition to those five evening flights (table 1) in zone B on which quantitative records were made, the writer has experienced many *Kerteszia* flights in B areas that were composed entirely of *homunculus;* and there is no doubt that in the wettest parts of zone B *bellator* is completely absent.

The Divergent Climatic Requirements of the Two Species in Their Area of Overlap

All the observations in this section were carried out in the Carapa-Eschweilera forests and the cacao plantations of Tamana which is in the C zone (fig. 1).

Methods. For observation of the vertical distribution of the mosquitoes, three series of platforms were built at distinct stations designated in the text as A, B, and C. The first two series (A and B) were built in the Carapa-Eschweilera forest, and C was built in an adjacent cacao plantation. The platforms in each series were built on or around a single tree at successive height intervals of ten feet. Each platform is

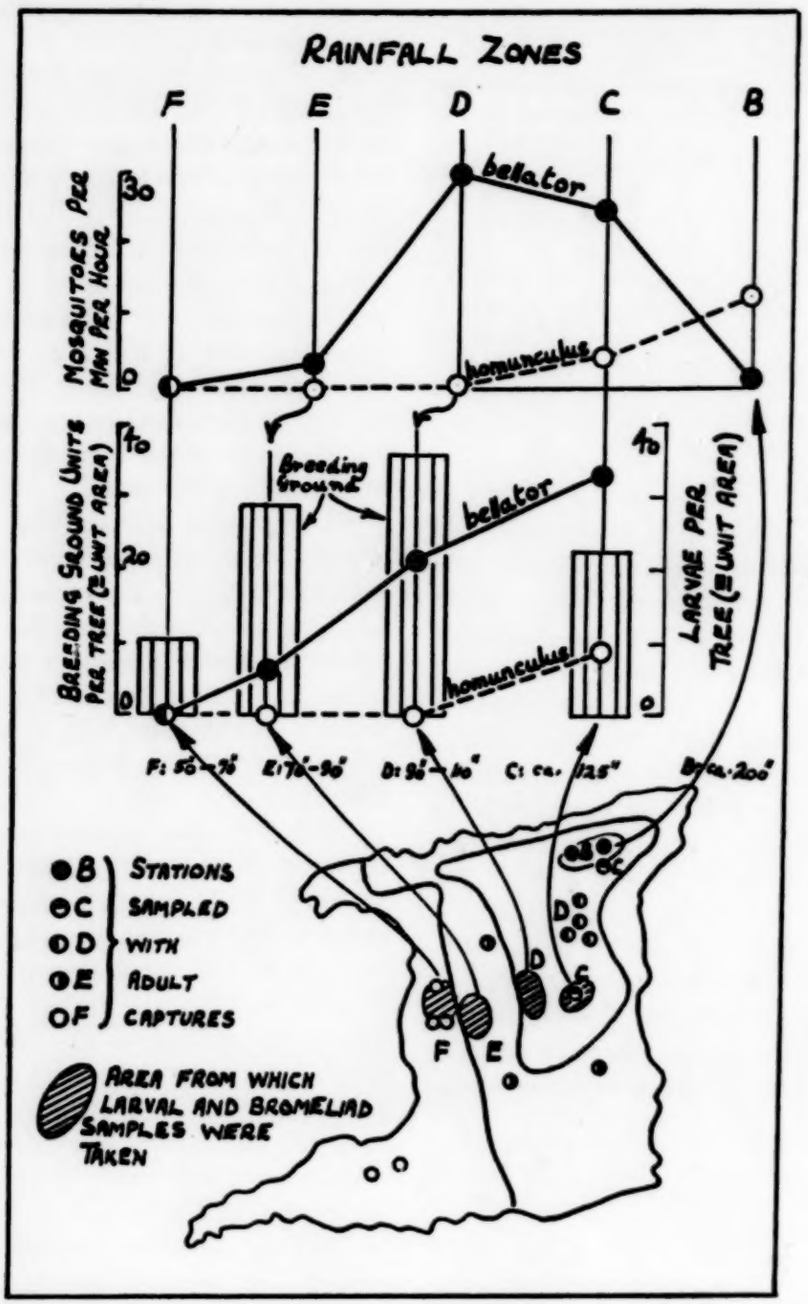


FIG. 1. The geographic variation of anopheline density (adult and larval data); its correlation with rainfall level; and its independence of the size of the total potential breeding ground (cf. table 1).

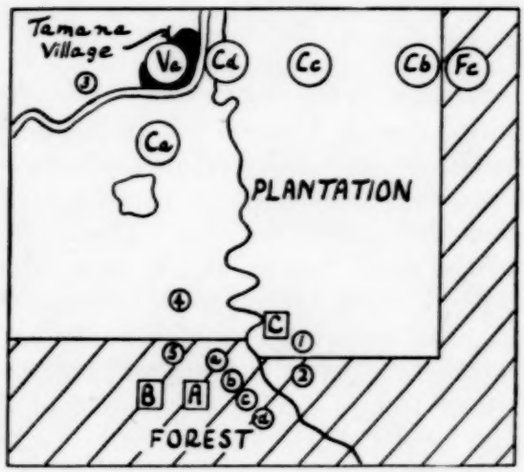


FIG. 2. Map of capture stations and breeding plots. A, B, and C are the three series of platforms. Ca, Cb, Cc, and Cd are ground level stations (adult captures) in plantation. 1, 2, 3, 4, 5; a, b, c, and d are breeding plots. Va is a house veranda.

designated by a combination of the station letter and the height. Thus, we have A5, A15 etc. to A55; B5 to B75; and C5 to C45. The A and B platforms stood in two markedly different pieces of forest. A was on a gentle slope where the trees were relatively low and open resulting in a heavy undergrowth. The B platforms were in a ravine where the trees were extremely tall. The forest was shadier than in A and freer of undergrowth. In addition to the forest platforms, other capture stations on the ground were used. The distribution of all is seen in figure 2.

The platforms were intended for the study of the vertical distribution of both breeding and feeding activities. They were 10 feet square. A small part of the total area was boarded over and used by mosquito catchers sampling the distribution of feeding. Only human bait was used. One man was stationed at each platform or ground station to be sampled and caught mosquitoes coming to feed on himself. Suction tubes were used. Three measures were adopted to minimize errors in sampling: (1) all catchers were tested against each other to exclude marked differences in attractivity to mosquitoes; (2) all catchers wore short trousers and had their arms bare while working. Mosquitoes were caught from the arm and leg; (3) one catcher never caught at the same platform on successive samplings. A regular rotation was maintained, possible differences in efficiency and attractivity being distributed throughout the range of levels sampled. Table 2 gives a list of the series of captures which comprise the present data.

A Weston light meter and a sling hygrometer were available at the beginning of the work and were used in obtaining the environmental data that accompany the mosquito data for capture series no. 2, and elsewhere. The light meter soon failed, and measurement of environmental factors was restricted to the use of a sling

TABLE 2. *List of mosquito capture series (feeding activity)*

CS	No. of days	Dates	Stations sampled	Capture mid-points
1	10	iii.28–iv.1.44	A5, A25, A55	10:00, 11;00 A.M., 12 Noon, 2:00, 3:00, 4:00 P.M.
2	9	viii.21–viii.31.44	A5, A15, A25, A35, A45, A55	(8:15, 9:15), (10:15, 11:15 A.M.) (12:15, 1:45), (2:45, 3:45), (4:45, 5:45), 7:15 P.M.*
3	5	ix.13–ix.21.44	A5, A55, Fa.	8:15 A.M. to 4:15 P.M., hourly
4	3	iii.7–iii.9.45	B5, B45, B75	1:15 to 8:15 P.M., hourly
5	4	iv.3–iv.9.45	C5, C25, C45	1:15 to 8:15 P.M., hourly
6	5	iv.26–v.1.45	B5, B15, B25, B35, B45, B55, B65, B75; C5, C15, C25, C35, C45	10:15, 11:15 A.M., 12:15, 1:15, 2:15, 3:15, 5:15, 6:15 7:15 P.M.
6a	3	v.2–v.4.45	B5, B25, B45, B65; C5, C25, C45	—as in CS6—
7	4	v.18–v.21.45	B5, B45, B75; C5, C45. Ca. Va.	—as in CS6—
8	5	vi.6–vi.13.45	Fc, Cb, Cc, Cd	—as in CS6—
9	3	viii.2–viii.7.45	B5, B25, B45, B65	—as in CS6—

CS = capture series.

All captures lasted ½ hr. except those marked (*) which lasted 1½ hr. Times given are capture mid-points. The number of days given is that of days actually spent in catching: the dates, however, are of first and last days in the series during which some days may have been lost due to rain, etc. All stations listed in a given capture series were sampled simultaneously. Captures grouped in brackets are lumped in table 4.

hygrometer when this was available. The light meter was used during capture series no. 2 as follows. The sensitive cell was exposed vertically at each observation by placing it on a fixed horizontal bench. Sun flecks were always avoided by sliding the cell horizontally across the bench. Every thirty minutes three readings were made thirty seconds apart. The entries in table 3 for the hourly light intensity are therefore means of 54 (6×9 days) readings. The noon light intensity in table 4 and figure 4 is underestimated, since in at least 50 per cent of the readings involved, the pointer passed beyond the calibration on the dial. This does not, however, affect the utilization of the data as their significance lies merely in the fact that the light changes are symmetrical about the noon high point. Humidity and temperature data given in table 4 and figure 5 were obtained with the sling hygrometer at the same time as light readings were made and are therefore similarly based on six readings per hour.

The total data obtained in this study are too bulky, even in summarized form, to reproduce here. Detailed summaries for all the capture series not given here are being deposited in the Princeton University Library and are available for inspection.

Activity cycles in relation to humidity. Tables 3 and 4 show for both species the changes in density (expressed as mosquitoes per man-hour of catching) that occur throughout the day. The activity cycle of both species is the type which Haddow (1945) refers to as "eocrepuscular": that is, activity is high in the early morning and early evening and falls to a distinct trough during the middle of the day. It was impracticable to make routine observations on the forest platforms after dark owing to the danger involved; this was done, consequently, in only two capture series, nos. 4 and 5. The two sets of data obtained from these observations (table 3) show that the increase of activity from 4 P.M. to 7 P.M. which was manifest in all the series, is followed by

TABLE 3. *Activity cycles for the capture series discussed in this paper*

		Capture midpoints												
		A.M.				P.M.								
		8:15	9:15	10:15	11:15	12:15	1:15	2:15	3:15	4:15	5:15	6:15	7:15	8:15
CS2	b	8.1	7.5	8.1	5.6	4.4	1.7	1.8	2.1	3.2	6.9		22.7	
	h	2.1	1.3	1.4	0.6	0.7	0.3	0.3	0.4	0.4	0.5		5.1	
CS4	b						7.6	4.7	7.1	5.3	4.0	61.6	101.5	30.2
(F)	h						3.1	2.4	1.3	1.1	0.7	4.7	25.5	13.3
CS5	b						2.3	1.2	1.2	2.7	4.0	37.2	164.8	15.2
(P)	h						0.5	0.5	0.3	0.7	1.3	4.5	17.1	7.1
CS6	b			10.0	5.4	4.9	2.9	1.9	1.5		2.4	21.2	124.8	
(F)	h			5.2	2.3	1.8	1.3	0.8	0.6		1.6	5.1	30.8	
CS6	b			8.5	5.8	2.8	3.4	3.6	3.8		11.6	56.0	146.0	
(P)	h			3.7	2.2	1.3	1.3	1.8	2.3		3.8	11.9	29.0	
CS6a	b			21.5	27.5	8.8	7.0	7.6	6.0		13.7	43.0	137.5	
(F)	h			17.8	12.1	5.7	4.5	3.7	2.1		5.5	8.7	54.8	
CS6a	b			6.3	7.8	4.4	4.7	3.3	3.8		13.3	69.1	69.4	
(P)	h			4.3	2.2	1.3	2.7	1.1	1.3		6.9	16.4	15.8	

Data on the other capture series have been deposited in the Princeton University Library. Entries in the table are mean densities for all levels sampled during the period, and expressed as mosquitoes per man per hour.

F = forest.

P = plantation.

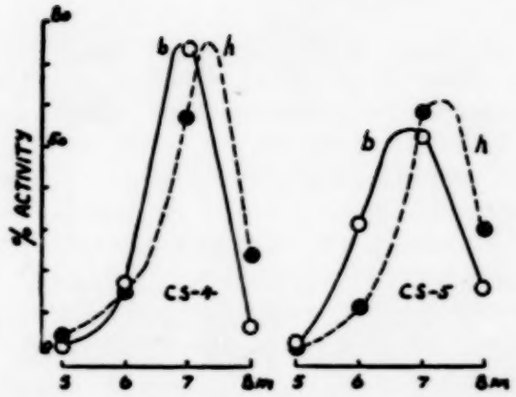


FIG. 3. Smoothed curves to illustrate the precession of *A. bellator* evening activity. Densities at 5, 6, 7, and 8 P.M. are expressed in per cent of the total for 5 P.M. to 8 P.M. Capture series nos. 4 and 5 (cf. table 3).

Taking the 3 day totals for CS no. 4 we have: 5:15 P.M. 18b, 3h; 6:15 P.M. 277b, 21h; 7:15 P.M. 457b, 115h; 8:15 P.M. 136b, 60h. Chi-square = 46; d.f. = 3; P, negligible.

Taking the 4 day totals for CS no. 5 we have: 5:15 P.M. 24b, 8h; 6:15 P.M. 223b, 27h; 7:15 P.M. 989b, 103h; 8:15 P.M. 91b, 43h. Chi-square = 63; d.f. = 3; P, negligible.

a sharp decline between 7 P.M. and 8 P.M. (fig. 3).

The trough of activity in the middle of the day reaches its lowest point well after noon: the majority of series show the lowest density during the 2:15 or 3:15 P.M. captures. This asymmetry of the activity curve about noon is an important feature, since it is highly indicative of the microclimatic variable in relation to which the distribution of mosquito activity is oriented.

Capture series no. 2 is the only series for which there are data on the microclimatic cycle for comparison with mosquito distributions (table 4 and fig. 5). There is clear asymmetry about noon in the activity curves for both *bellator* and *homunculus*. Of the three microclimatic variables studied, light shows the least relationship to the mosquitoes' activity: the light changes are completely symmetrical around the noon high point. This is not the case, however, for either temperature or humidity: both show a marked lag behind light, and reach their highest and lowest points respectively in the early afternoon. It is the humidity curve which is most clearly related to the activity cycle of the anophelines.

The daily lag of temperature and humidity changes behind the movement of the sun is a familiar meteorological effect. Its extent, as Haddow (1945), for instance, has noted, is accentuated in dense vegetation. The present mosquito data give some indication that, as would be expected on this view, the lag is less marked in open vegetation. In capture series nos. 6, 6a, and 7, captures were made throughout the day simultaneously in forest (the B platforms) and in the more open cacao plantation (the C platforms). In all three series the midday low-point of activity in cacao precedes that in forest (table 3). This is particularly clear in capture series no. 6 (fig. 4) in which the largest samples were taken. It is clear that while density of vegetation

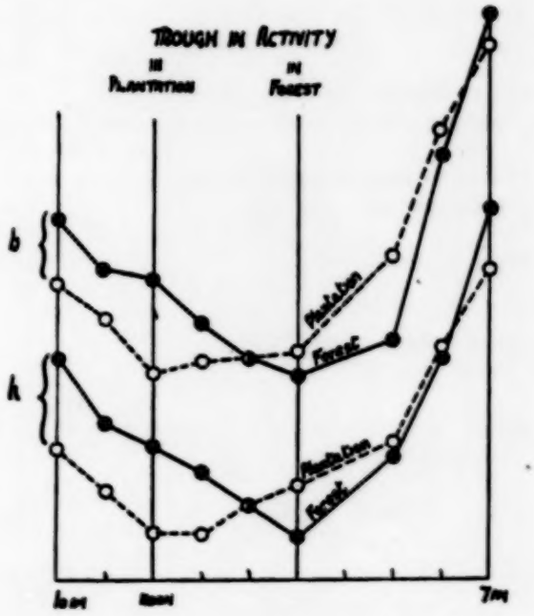


FIG. 4. Difference in activity cycles in forest and plantation: the midday trough and late afternoon rise in activity occur earlier in the plantation than in the forest. Each curve is a log plot of density (in mosquitoes per man-hour); densities from table 3. The points of lowest density in forest and plantation have been equated to facilitate comparison of the vegetation types; for the same reason the two species are treated separately.

b = *bellator*, h = *homunculus*.

TABLE 4. *The vertical and temporal distribution of mosquito biting activity in relation to microclimate in forest*

Capture midpoints		A.M.		P.M.				Totals
		8:45	10:45	1:00	3:15	5:15	7:15	
		Anopheles homunculus						
Vertical distribution (in per cent)	55′	8	2	0	0	12	45	*32*
	45′	16	11	5	5	12	14	13
	35′	*27*	12	5	10	27	13	15
	25′	17	18	28	16	7	12	14
	15′	19	*30*	*48*	*53*	15	9	15
	5′	13	27	14	16	27	7	11
		Anopheles bellator						
	55′	23	17	7	20	22	*34*	*38*
	45′	*38*	25	26	*25*	*23*	24	26
	35′	23	*28*	*33*	21	21	15	10
	25′	8	12	19	18	12	11	11
	15′	5	15	12	11	15	11	11
	5′	3	3	3	5	7	5	4
Total mosquitoes caught	b	373	371	132	101	272	1837	3086
	h	83	56	21	19	26	411	616
Mean density (in mosquitoes per man hour)	b	7.8	7.2	2.9	2.0	5.2	22.7	
	h	1.7	1.1	0.5	0.4	0.5	5.1	
Per cent distribution of mean densities	b	16	15	6	4	11	48	
	h	18	12	5	4	5	55	
Mean height (in feet)	b	40.6	36.0	33.8	35.6	35.3	39.4	
	h	28.8	24.1	18.6	18.4	26.6	40.5	
Mean light intensity at ground level (units)		11.3	28.9	35.0	21.3	7.9	0.6	
Mean temperature (° F.) at ground level		77.0	79.8	80.7	80.3	80.0	77.3	
Mean relative humidity at ground level		93.4	90.1	88.1	88.2	92.4	96.3	

CS2, 9 days catching.

b = *bellator*, h = *homunculus*.

The significance of the morning and evening differences between the activity cycles of the two species has been tested. The morning difference is not statistically significant in the present data, but the evening difference is overwhelmingly so. Taking the 9 day totals for each species in the separate capture periods listed in table 2 we have: 3:45 P.M. 57b, 12h; 4:45 P.M. 86b, 12h; 5:45 P.M. 186b, 14h; 7:15 P.M. 1837b, 411h.

Chi-square = 17, d.f. = 3, $P = \ll 0.01$; with the largest contribution to chi-square at 5:45 P.M. See also table 5.

wil not affect the symmetry of the light curve, it will affect the humidity curve; and the discrepancy between the activity cycles in forest and in plantation constitutes corroboration of the conclusion that the midday depression of mosquito activity is related to humidity changes.

Vertical distribution in relation to activity cycle and humidity. The distribution of the two anophelines as shown in table 4 is by no means random on the vertical gradient of microclimate within the forest. It is evident that the pattern of vertical distribution for each species undergoes a cycle of shifts throughout the day and this cycle follows the curve of total mosquito density changes, i.e., the activity cycle (fig. 5). In both species the vertical distribution of activity shifts downwards in successive periods throughout the morning and early afternoon, matching the decrease in total activity. The important feature to be noted is that the lowest distribution of activity on the vertical gradient of microclimate, like the lowest point on the activity cycle curve, occurs well after noon.

This is clear evidence that the shift of mosquito activity downward on the gradient of forest microclimate is, like the changes in total density (the activity cycle), related to the decrease in humidity. Were the vertical distribution of the mosquitoes an orientation primarily to the light gradient, the downward shift should cease immediately after noon. As it is, it does not cease—and this is particularly clear in the case of *homunculus*—until after 3 P.M. when humidities begin to rise.

The upward shift of the distribution continues, on most evenings, until activity is concentrated in the highest levels sampled. Thus, during the crepuscular period when the density of feeding mosquitoes is highest, their activity is distributed overwhelmingly in the canopy of the forest. At this time the whole forest profile is evidently near saturation (cf. table 1 in Pittendrigh, 1948).

TABLE 5. *Total mosquitoes caught, the interspecific ratio, and the precession of* bellator *activity in the evening*

CS	Total mosquitoes caught		h/b	Per cent caught after 5 P.M.	Rate of increase of density	
					6 P.M./5 P.M.	7 P.M./6 P.M.
	b	h		b > h	b > h	b < h
1	201	68	.34			
2	3086	616	.20	66 < 69	2.2 > 1.1	3.3 < 9.8
4	999	235	.24	89 > 85	15.4 > 7.0	1.6 < 5.5
5	1371	193	.14	97 > 94	9.3 > 3.4	4.4 > 3.8
6 (B)	3458	964	.36	86 > 78	9.0 > 3.2	5.9 < 6.1
6 (C)	2994	706	.24	89 > 79	5.0 > 3.2	2.6 > 2.4
6a (B)	1593	654	.41	73 > 63	3.2 > 1.6	3.2 < 6.3
6a (C)	810	228	.28	84 > 77	5.2 > 2.4	1.0 = 1.0
7 (B)	1369	345	.25	70 > 61	3.5 > 1.5	0.9 < 2.6
7 (C)	543	127	.24	88 > 81	8.9 > 1.1	1.6 < 3.0
7 (Ca)	99	25	.25	81 < 92	13.2 > 2.2	0.9 < 1.1
7 (Va)	39	3	.08	95 < 100	∞ > 0.0	2.4 > ∞
8 (Fc)	157	96	.60	89 > 45	7.5 > 1.4	2.0 < 2.6
8 (Cb + c)	374	100	.30	91 > 89	8.6 > 5.3	2.2 < 3.0
8 (Cd)	622	81	.10	80 > 57	3.0 < 3.5	3.9 > 2.0
9 (B)	2381	677	.29	56 > 52	1.8 < 3.5	1.3 < 2.3

See text for detail.
b = *bellator*.
h = *homunculus*.

TABLE 6. *The differential elimination of* A. homunculus *in open vegetation (cacao plantation)*

	Total mosquitoes caught				h/b	Time of onset of *bellator's* evening activity
	Forest		Plant'n		f > p	f > p
	b	h	b	h		
April 26	1122	240	610	86	0.21 0.14	6:30 5:45
27	763	264	642	196	0.35 0.31	5:45 4:45
28	301	80	522	85	0.27 0.16	6:45 6:00
29	287	104	597	164	0.36 0.27	6:00 5:45
May 1	985	276	623	175	0.36 0.36	6:00 4:45
2	517	210	299	86	0.41 0.35	5:45 3:45
3	611	257	265	78	0.42 0.29	4:15 5:00
4	465	187	246	64	0.40 0.26	5:15 4:15

Capture Series 6 and 6a.
b = *bellator*.
h = *homunculus*.

Comparing the h/b fraction in forest (f) and plantation (p), it is higher in forest with a mean difference (f − p) of 0.08 ± 0.014 from which $t = 5.9$, d.f. = 7, $p = < 0.01$. See footnote no. 8.

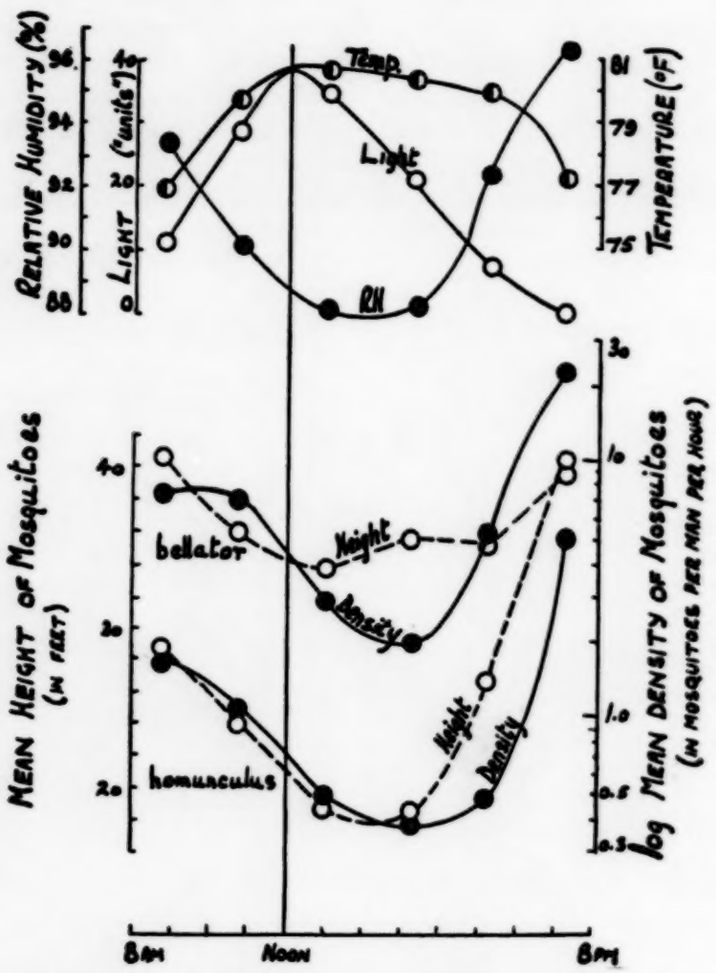


FIG. 5. Cycle of microclimate, mosquito densities, and vertical distribution. Capture series no. 2 (cf. table 4).

The interspecific difference in humidity requirement. The general correlation of activity cycle and vertical distribution with the humidity curve applies to both species. There is, however, a clear interspecific difference between the anophelines in their response to the humidity changes.

In capture series no. 2 (table 3) which is the only series that samples the early morning period between 8 A.M. and 10 A.M., the initial drop in humidity during this period is followed by a sharper drop in the density of *homunculus* than in that of *bellator*. Conversely, the increase in densities in the late afternoon occurs later in the case of *homunculus* (fig. 5). Figure 3 shows particularly clearly the lag of the evening peak of *homunculus* activity behind that of *bellator*.

The fact that the initial increase of *homunculus* densities occurs at a later (wetter) period than that of *bellator* is evident throughout the data for the other capture series. In table 5 the rate of increase of the density of the two species from 5 P.M. to 6 P.M. and 6 P.M. to 7 P.M. is given as two simple factors. It is evident that during the earlier interval (5 P.M. to 6 P.M.) *bellator* densities increase at a greater rate than do those of *homunculus*. Conversely, in nearly all cases, *homunculus* increases faster during the later interval.[7]

[7] There is a feature of the statistics given in table 5 which requires comment. Expressing the activity of each species between 5 P.M. and 7:30 P.M. as a per cent of their respective total activity throughout the entire period sampled, it is found that the *bellator* per cent exceeds that for *homunculus*. On the face of it this might suggest that *bellator's* activity is relatively more reduced than *homunculus'* by the low midday humidity. This is not a valid conclusion however. The difference between the species in the per cent value of their 5 P.M.–7:30 P.M. activity is due to the delay of *homunculus* behind *bellator* in rising to its evening maximum; this coupled with the cessation of sampling at 7:30 P.M. before the evening flight is finished, leads to an underestimation of the *homunculus* evening activity relative to that of *bellator* and a consequent overestimation of its (*homunculus'*) midday activity.

The two species differ in their vertical distributions even more markedly than they do in their activity cycles. It is abundantly clear throughout all capture series that, with the significant exception of the evening period, *homunculus* occurs at lower levels than *bellator* (table 4; fig. 5). The exception of the evening period (fig. 8) is significant because it is the only period of all those sampled when the gradient of microclimate within the forest is eliminated: the two species differ in their relation to one of the microclimatic variables.

Now it has already been seen that the activity cycle and the cycle of shifts of vertical distribution are both correlated with humidity rather than with light, in that both are asymmetric about noon. The interspecific difference is then interpreted as a difference in humidity requirement. Thus, as humidity falls in the morning *homunculus* densities fall sooner than those of *bellator* and the shift in *homunculus* vertical distribution not only occurs sooner and proceeds further, but persists longer; that is, like the rise of the activity curve in the evening, the upward shift of distribution occurs later (when humidities are higher) in the case of *homunculus* than in *bellator* (fig. 5).

The differential elimination of A. homunculus *in open vegetation.* It follows that if the pattern of relative abundance of the two anopheline species on the forest profile is a function of the graded humidity then this pattern should obtain in comparing their distributions in distinct vegetation types whose differing structures determine differing overall humidity. Forest and the much drier plantation may be compared with this in view.

The first comparison concerns the relative total density of the two species. Table 6 lists the *homunculus* densities relative to those of *bellator* (as a fraction h/b) for each day and each vegetation type separately throughout capture series 6 and 6a which comprised simultaneous samples in both forest and plantation. It is seen that the fraction is

TABLE 7. *The differential elimination of* A. homunculus *in plantation and village, and the evening shift in the distribution of activity*

A. Vertical distributions in per cent. Forest.

		5 ft.	45 ft.	75 ft.	Total caught
Day	b	26	52	22	410
	h	67	21	12	129
Evening	b	29	35	36	959
	h	35	36	29	216

B. Horizontal distributions in per cent. Forest, plantation, and village ground stations.

		B-5	C-5	Ca	Va	
Day	b	64	23	11	1	246
	h	80	19	2	0	146
Evening	b	45	36	13	6	542
	h	44	36	17	3	96

CS7. Forest samples were taken on the B platforms; plantation samples on the C platforms (C-5) and at a more exposed site, Ca; Va is a veranda of a house in the village; cf. figure 1.

consistently lower in the plantation which is the drier vegetation type. The difference though slight is highly significant: the probability of these repeated differences being due to chance is less than 0.01.[8]

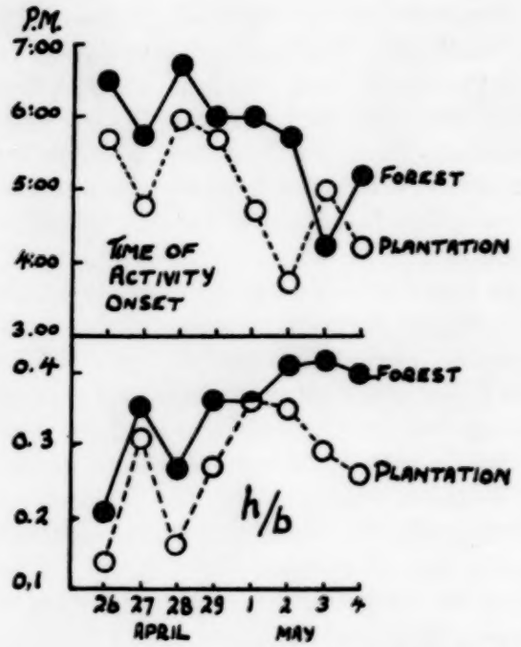


FIG. 6. The onset of mosquito evening activity (*bellator* used as indicator) and the size of the *homunculus/bellator* ratio in forest and plantation. Capture series 6 and 6a. Activity onset is arbitrarily taken as the time at which activity has reached 10% of maximum; it occurs earlier in the plantation and is negatively correlated with the size of the *homunculus/bellator* ratio which is consistently lower in the plantation (cf. table 6).

The second analogy that exists between forest-plantation distributions and ground level-canopy distributions concerns the shift that takes place with the saturation of all environments in the evening. In capture series no. 7 (table 7) samples were taken simultaneously at three levels in the forest (B platforms), two levels in plantation near forest (C platforms), in more exposed plantation (Ca) one half mile away from forest near the village of Tamana, and in the open veranda of a house (Va) in the village. The densities of both species fall at successively drier stations on the horizontal gradient, and it is seen that *homunculus* densities fall faster than those of *bellator*. The feature of these captures with which we are now concerned is the change that takes place

[8] In making this comparison care has been taken to determine whether or not the difference is due to peculiarities of sampling. Should the onset of the evening activity occur later in plantation than in forest, the known lag of the *homunculus* curve behind that of *bellator*, and the fact that sampling stopped before activity was over, would produce the very effect we are interested in, viz., a drop in the plantation of *homunculus* densities relative to those of *bellator*. Figure 6 plots the onset of evening activity (*bellator* used as indicator) in forest and plantation on each day of both capture series 6 and 6a. A density of 10 per cent of the maximum density is arbitrarily taken as an indicator of activity onset. The time at which this density was reached is interpolated in the activity cycle data. It is seen that there is considerable daily change in the timing of activity; however, while there is a constant difference between the vegetations, it is the plantation which precedes the forest so that the lag of *homonculus* will, if anything, have led to an underestimation rather than to an overestimation of the effect with which we are concerned. It is also interesting to note in figure 6 that there is a general correlation between the time of activity onset and the size of the h/b fraction: the earlier activity begins, the larger does h/b become, since the less does the cessation of sampling at 7:30 P.M. lead to an underestimation of the *homunculus* evening flight.

during the evening period of maximum activity. At this time with the loss of the humidity and temperature gradient the distribution of both mosquitoes shifts upward into forest levels that were too dry in the middle of the day; and the two species closely approximate each other's distribution. Table 7 shows that as this happened on the vertical gradient there was a similar shift outwards on the horizontal gradient to the cacao and village, resulting in a closer approximation of the *bellator* and *homunculus* distributions than obtained by day.

The effect of vegetation structure is also amply illustrated by comparison of data from the A and B platforms. Thus h/b fractions from the A platforms larval data (table 8) are much lower (.09, .06, and .22) than those from the B platforms (.69, .44, and (1.09)) which are in much more protected, taller forest. Moreover, in the B platforms *homunculus* shifts higher up the profile and further overlaps *bellator* than it does at A.

Humidity and the Availability of the Breeding Ground

It has been shown that in their area of overlap the two species are distributed significantly differently with respect to microclimate. The analysis of activity cycle and vertical distribution in capture series no. 2 leaves little doubt that humidity is the variable to which activity is adaptively oriented. Further, the difference between the species is in relation to humidity. Thus the initial hypothesis of an interspecific difference in humidity requirement, derived from the geographic distribution pattern of the two species and the distribution of precipitation is, in its general terms, upheld (fig. 7).

It remains to consider the possible mechanisms whereby the geographic distribution of rain could bring about the observed changes in mosquito density. In essence the problem is how does declining rainfall reduce population size since first one then the second species shows a steady decline of density as rainfall level decreases?

Table 1 immediately eliminates one obvious possibility, namely that the decline in the density of mosquitoes is a secondary effect caused by a diminution of the bromeliad flora. Actually the size of the total potential breeding ground per unit area is higher in zone D than zone C although the larval density per unit area

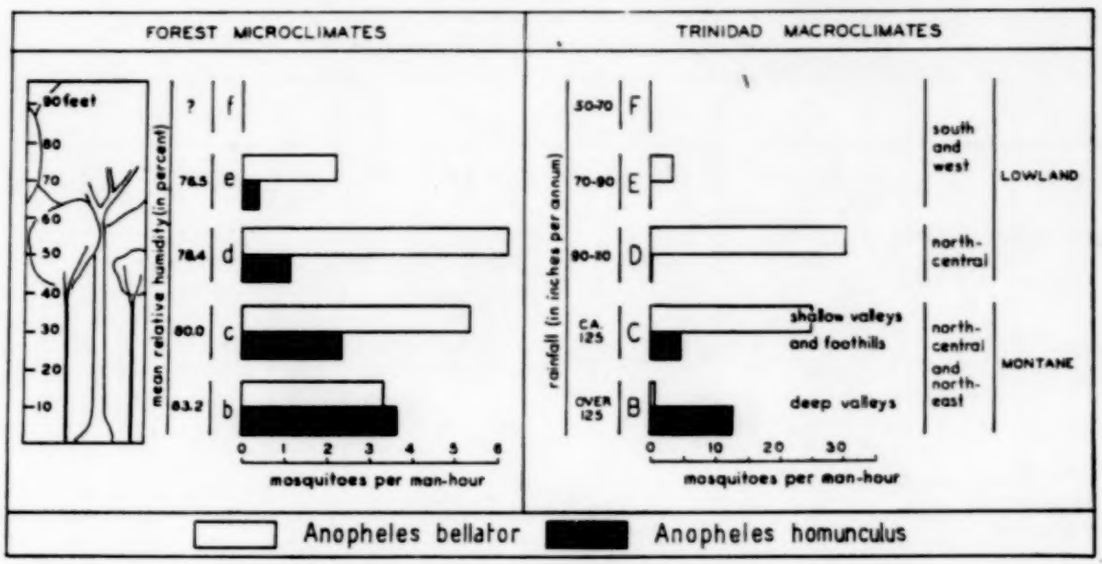


FIG. 7. The identity of distribution pattern of the two anophelines on a) the vertical gradient of humidity in forest in the middle of the day, and b) the geographic gradient of precipitation levels.

a) is based on capture series no. 6 from 10 A.M. to 4 P.M.; levels have been lumped.

b) is based on table 1.

(as well as per plant) falls significantly in the drier zone. And in zone E, which is on the periphery of *bellator's* range, densities of this species are down to one-fifth of those in zone C in spite of the fact that the total potential breeding ground is larger in the drier zone. It is clear therefore that decreasing rainfall effects a reduction of mosquito density independently of its (the rainfall's) effect on the bromeliad flora.

The vertical distribution of anopheline breeding activity in the Tamana forests provides clear indication of how precipitation level may affect mosquito densities even though the total potential breeding ground (the bromeliad flora) remains undiminished.

Breeding activity was studied in all three platform series as follows. The greater part of the available space on each platform was taken up by a latticework which supported in its interstices a standard plot of bromeliads. These standardized plots (see table 8 for details) at all levels on a platform series gave a measure, through the larval population recovered at the end of a month from each level, of the vertical distribution of breeding activity.

Table 8 gives the results of the six samples that were obtained in this way. It is very clear that the interspecific difference in vertical distribution which was found to hold for feeding activity obtains for breeding: *homunculus* larvae occur at consistently lower level than those of *bellator*. Moreover the difference between the species is here at least as great as the maximum (midday) difference found in feeding activity (fig. 8).

This divergence in breeding ranges is of considerable importance since the bromeliad flora has itself a well-defined, nonrandom, distribution on the forest profile. Elsewhere (Pittendrigh, 1948) it has been demonstrated that the bromeliad population in general is concentrated in the canopy levels of the forest because of its light demand. There is, within the flora, a segregate of bromeliads that are tolerant of shade and penetrate the lower forest levels, but these are all small sized species (cf. fig. 2 in the following paper). It is therefore apparent that in their breeding activity the two mosquitoes encounter very different fractions of the total bromeliad flora in the C zone forests. *A. bellator* has access to the whole flora, and its zone of maximum breeding activity

TABLE 8. *The vertical distribution of breeding activity in forest*

		A-Platforms						B-Platforms					
Date		Jun.-44		Sep.-44		Nov.-44		Mar.-45		Apr.-45		Jun.-45	
Composition of plot		10Ga 10Gl		10Ga 10Gl		10Ga 10Gl		10Ga 10Gl		10Ga 10Vp 10Gl		10Vp	
Species		b	h	b	h	b	h	b	h	b	h	b	h
Total larvae		2549	220	1734	111	545	122	324	224	625	277	164	176
Vertical distribution in per cent	65 ft.							22	7	10	2	35	4
	55 ft.	9	0	7	0	9	0	18	1	10	3	11	8
	45 ft.	27	0	11	0	15	0	27	7	21	11	13	6
	35 ft.	27	0	16	0	24	0	9	12	22	19	18	18
	25 ft.	22	13	27	17	23	6	11	26	24	26	6	20
	15 ft.	10	16	33	39	28	36	8	25	9	22	13	21
	5 ft.	5	71	6	44	1	59	5	22	4	17	4	23
	h/b	.09		.06		.22		.69		.44		1.09	

b = *bellator*, h = *homunculus*, Ga = *Gravisia aquilega*, Gl = *Guzmania lingulata*, Vp = *Vriesia procera*.

TABLE 9. *The bromeliad flora below 25 feet in forest; and its anopheline larval population*

Bromeliad species	M. Ht.	N	M. W. C.	b	h	h/b
Hohenbergia stellata	20′	15	326	16	8	0.5
Tillandsia monadelpha	19′	20	8	0	0	
Tillandsia anceps	14′	15	10	0	0	
Guzmania lingulata	11′	100	65	14	53	3.8

Sample transect approximately 800 feet long, 20 feet wide; b = *bellator;* h = *homunculus;* M. Ht. = mean height above ground; N = number of plants; M. W. C. = mean water content in cc.

(as sampled on platforms independently of the naturally occurring bromeliads), which is in the upper levels, coincides with the heaviest natural growths of these plants. On the other hand *homunculus* is restricted by its microclimatic demands to forest levels that support the thinnest bromeliad population composed exclusively of the small shade tolerant species that are poor water holders.

Guzmania lingulata, Vriesia simplex, Vriesia longibracteata, Tillandsia anceps, and *Tillandsia monadelpha,* are throughout the whole of Trinidad the only species of bromeliads which regularly exploit the lower levels. In some areas such as Tamana, *Hohenbergia stellata* a large species, occasionally gets down into the *homunculus* oviposition range, but this plant is never common. Table 9 gives the larval population found in all the bromeliads examined below 25 feet in a strip of forest near the B platforms. *Hohenbergia stellata* was unusually common in this sample. Even so it was only ten per cent of all the plants found; it had the highest average elevation above ground of all the species and only 35 per cent of its larval population was *homunculus. Guzmania lingulata* was the commonest plant; it occurred on the average nine feet lower than *Hohenbergia stellata*

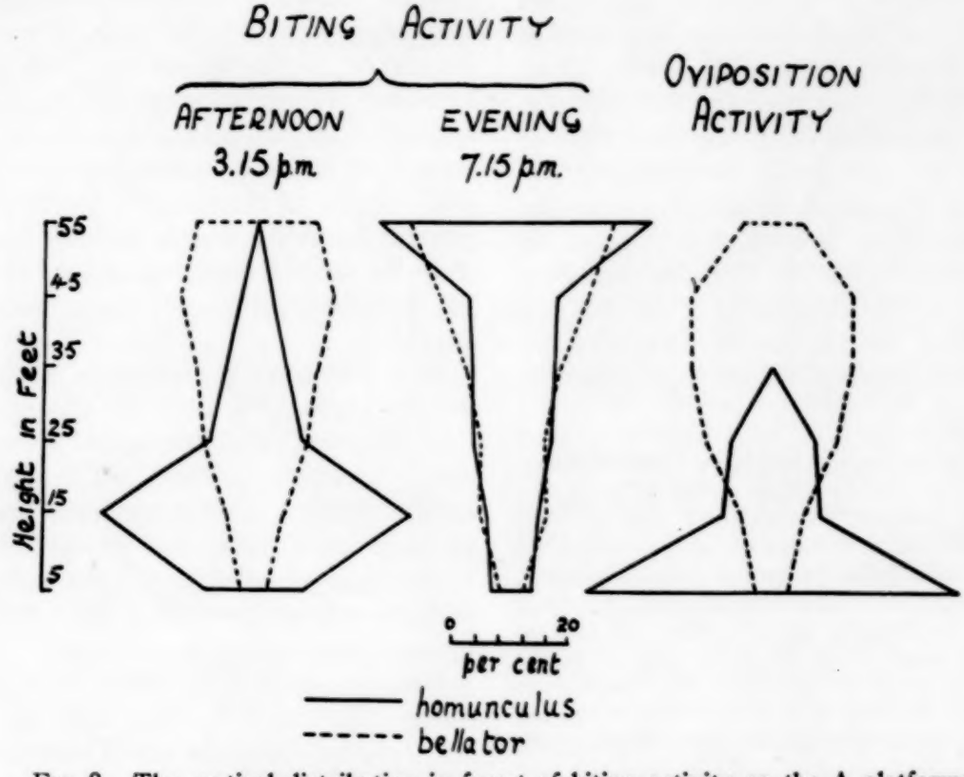


FIG. 8. The vertical distribution in forest of biting activity on the A platforms in the mid-afternoon (3:15 P.M.) and in the evening (7:15 P.M.), and the vertical distribution of oviposition on the A platforms in June 1944.

Biting activity data from table 4 (CS2).

Oviposition data from table 8.

and here *homunculus* formed almost 80 per cent of the recovered larvae.

Considering all the data obtained in the study area in Tamana, zone C, the true relative densities of the two species (i.e., densities based on samples covering the whole range of microclimate in the forest) is such that the fraction total *homunculus*/ total *bellator* is much less than 0.5; compare tables 5 and 8.[9] It seems obvious that the differential exclusion of *homunculus* from the main breeding ground in the canopy, consequent upon its humidity demands, is responsible for the well marked difference in total density between the species. In other words, the numerical superiority of *bellator* is to be associated with the fact that its vertical breeding range coincides with the densest bromeliad population which includes all of the large species.

The conditions in the much wetter B zone point up fully the situation just described for Tamana. In the B zone valleys *homunculus* completely dominates the *Kerteszia* population—the h/b fraction here is between 12 and 27 (table 1) as compared with less than 0.5 for Tamana. This is associated with the fact that in addition to the small bromeliads, *homunculus* in zone B is utilizing fully the large bromeliad species which occur in those microclimatic niches that are here not only fully illuminated (the limiting requirement for the plants) but very humid (the limiting requirement for *homunculus*).

In the B areas it would have required elaborate platform studies which owing to their cost were not possible, to determine directly that *homunculus* is in the canopy of the forests of this zone as is being suggested here. However, it is known that in the drier C zone it is only *bellator*, the canopy species, which is able to utilize adequately the breeding ground in the exposed plantation. Similarly in openings in the forest in zone C *bellator* dominates the *Kerteszia* captures at ground level because, owing to the opening of the forest, the microclimate near the ground is changed so as to simulate that of the canopy. Table 10 gives captures illustrating this. Now, in the B areas, *homunculus* dominates the population both in plantations and in openings in the forest just as *bellator* does in C; and like *bellator* in C, *homunculus* in B becomes scarce at ground levels inside the forest immediately adjacent to those openings where it is common. The inference that *homunculus* is in the canopy of intact forest in B seems fully valid.

[9] July readings on the B and C platforms gave h/b values greater than 1.0; these are highly significant exceptions caused by a peculiarity of sampling procedure discussed fully in the subsequent paper of this series.

TABLE 10. *The relation between the* Kerteszia *flight in forest clearings and adjacent intact forest*

	A55	A5	Fa
bellator	268	12	328
homunculus	53	106	91
h/b	0.2	8.9	0.3

A55 in the canopy, A5 on the ground inside the forest, Fa in a wide clearing 20 yards from A5. Capture series no. 3. Four days' totals, 8:30 A.M. to 4:00 P.M.

The facts are, then, that in zone B high general humidity conditions permit *homunculus* to enter the fully illuminated vegetation niches where the bulk of the breeding ground is concentrated; here the species is at its maximum density. In zone C it is forced to darker (more humid) levels to compensate for the lower general humidity and is thereby divorced from the light-demanding bromeliad flora; the population becomes correspondingly smaller.

It is necessary therefore to distinguish in any given area between the apparent (or potential) and the actual (or available) breeding ground. The latter is only a fraction of the apparent breeding ground, since this—the bromeliad flora as a whole—is distributed through a far wider range of humidities than the mosquitoes can evidently tolerate.

Finally, it is noteworthy that this is nicely illustrated by the data available for *bellator* breeding in Tamana cacao plantations, which have been presented in detail earlier (Pittendrigh, 1950a). Figure 9, based on data in the earlier paper, is a histogram showing the vertical

distribution of breeding ground units (cf. p. 45) on the profile of plantation. It has the vertical distribution of *bellator* oviposition superimposed on it. What is significant for the present discussion is that the breeding activity of the mosquito is distributed significantly lower than the breeding ground as a whole (chi-square is 24; d.f. = 1; $P < 0.01$). Even here, therefore, in the wettest zone in which *bellator* is common in plantations, densities are not limited by the size of the total potential breeding ground, but by the availability of this being reduced by low humidity in the higher plantation levels where the bromeliads are concentrated. It then becomes clear how *bellator* densities themselves will continue to fall in successively drier geographic zones in spite of an increasingly large bromeliad flora, since the availability of this diminishes progressively as the upper strata of plantations become progressively less humid.

In the foregoing discussion we have been principally concerned to show how humidities below the optimum range render potential breeding grounds inaccessible. It is evident from the microdistributions of activity discussed earlier that *bellator's* optimum humidity range is lower than that of *homunculus:* it not only tolerates lower humidities than its sister species but requires them insofar as its densities are concentrated spatially and temporally into drier conditions. Whereas in the drier sections of the island the interspecific difference in humidity range places *homunculus* at an adaptive disadvantage in exploiting the microclimate surrounding the principal breeding ground the converse holds in the wettest areas. There the high humidities are evidently above the *bellator* optimum and densities of this species drop almost to zero while those of *homunculus* rise. While decline of the general humidity level can be offset by a compensatory downward movement on the forest profile it is clear that there is no such recourse (now an upward movement) open to the insect when humidities rise above the optimum, at which point the mosquito is already in the highest (driest) canopy levels.

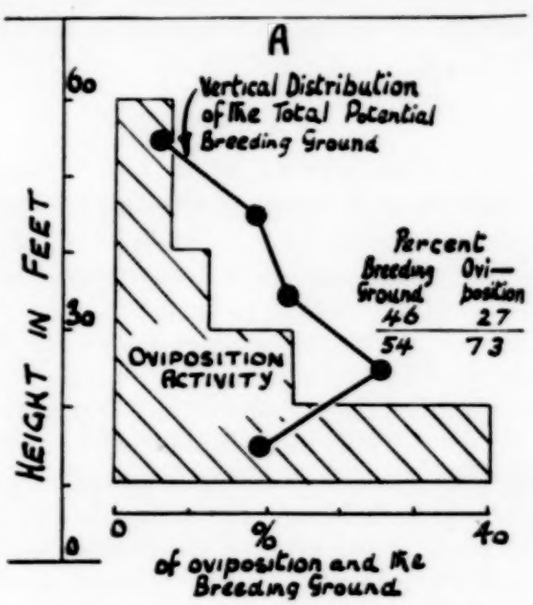


FIG. 9. The non-coincidence of the vertical distribution of the total potential breeding ground and of *A. bellator* oviposition in a cacao plantation in Tamana (zone C).

From the data given by Pittendrigh, 1950a.

Comparing breeding ground units above and below 30 feet with oviposition above and below 30 feet in a contingency table we get chi-square = 24; d.f. = 1; $P < 0.01$.

DISCUSSION AND CONCLUSIONS

It will come as no surprise to the insect physiologist if the conclusion is drawn from an ecological study that the water-relations of the environment constitute the prime climatic variable in relation to which mosquito activity and distribution are oriented. Wigglesworth (1946) remarks that it "is very largely true to say that the physiology of the insects is the story of their water relations." "An incessant struggle for water is reflected in every aspect of their organization."

The present studies were carried out in circumstances that precluded the highly desirable parallel physiological analysis that could have demonstrated experimentally the existence of a difference between the species in their behavior towards and tolerance of diverse

humidity and temperature conditions. Nevertheless the inference that humidity is of prime importance in this case seems well founded on a multiplicity of correlations. (1) First, the shape of the biting cycle curve through the daylight hours parallels the humidity curve extremely closely. (2) This is equally true of the downward shift in vertical distribution which is most depressed during the driest period of the mid afternoon. (3) We are thus led to the conclusion that the midafternoon vertical distributions of the two species are oriented with respect to the humidity gradient as such. And this conclusion receives striking corroboration from the way the interspecific difference on the vertical humidity gradient is matched by the interspecific difference on the geographic gradient of precipitation level. (4) The other, implicit, conclusion that the two species differ in their water relations leads us to expect a difference between their respective biting cycle and vertical-shift curves in respect of shape and precise timing since these have already been inferred to be humidity determined. This prediction is met in the earlier decline of the densities and elevation of *homunculus* in the morning and their delayed rise in the evening.

While there can be little doubt that the form of the biting cycle curve and the midday elevations are adaptively related to humidity the writer does not wish to imply that this factor alone serves for the immediate determination and orientation of activity. Lack (1949), in considering bird distributions, notes the necessity "to distinguish between the proximate factors concerned with internal mechanism and the ultimate factors concerned with survival value." Where there is a correlation, albeit a complex one, between light, temperature and humidity, the variable that functions for the insect as its indicator of environmental conditions—and acts therefore as the proximate stimulus to activity—may well be that for which it has greatest perceptual acuity, irrespective of whether or not it is the really limiting variable in terms of the species' particular adaptations.

In the case of diurnal activity cycles there are specific grounds for wariness in the inference of actual "determinants" because in many cases intrinsic physiological rhythms are known to be involved (Welsh, 1938). Gunn (1940) has, for example, recently demonstrated that the cockroach activity cycle persists for several days in the absence of cyclical environmental change which evidently functions to a large extent as a mere "trigger" for the intrinsically determined rhythm. And Bates (1947) has reported a very remarkable experiment which, in a parallel fashion, suggests that vertical distributions may be proximately determined by factors other than those to which the distributions are ultimately oriented. He showed that on the wall of an experimental cage in which there were no detectable gradients either of light, temperature, or humidity, the three species *Haemagogus spegazzini, Aedes serratus,* and *Trichoprosopon digitatum,* assumed the same pattern of vertical distribution as they do in forest.

Summary

(1) The activity cycle and vertical distribution in forest of the two bromelicolous anophelines in Trinidad (*A.* (*K.*) *bellator* and *A.* (*K.*) *homunculus*) are oriented in relation to humidity. As the density of the anophelines falls with the decrease of humidity in the morning, the mosquitoes shift their vertical distribution downward into more humid levels of the vertical climatic gradient in forest. Conversely, as humidity and mosquito density increase in the evening the activity shifts upward into the highest canopy levels.

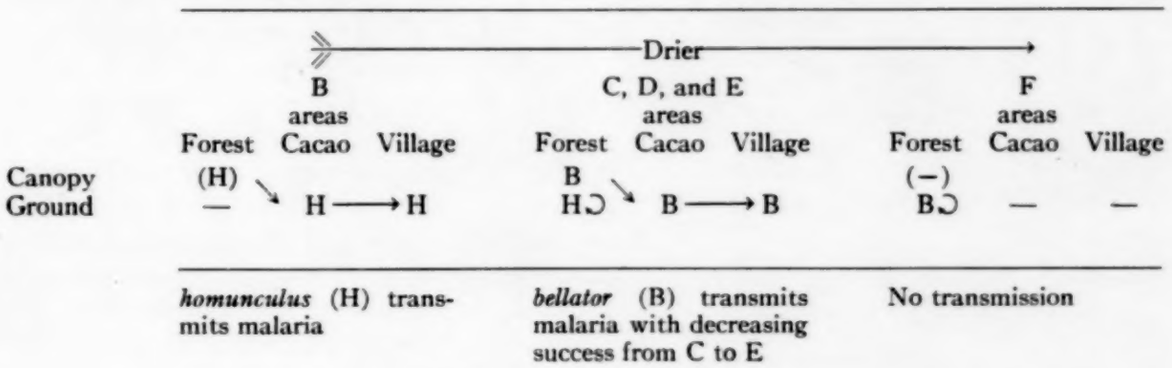
(2) There is an interspecific difference in humidity requirement between *bellator* and *homunculus* which is manifested in the following ways: (1) the morning drop in *homunculus* activity precedes that of *bellator;* (2) the evening rise of *homunculus* lags behind that of *bellator;* (3) *homunculus* occurs at lower forest

levels than *bellator* when a humidity gradient is created in the forest in the middle of the day; (4) *homunculus*' vertical distribution remains at its lowest longer than that of *bellator* in the afternoon; (5) *homunculus* is differentially eliminated in dry, open vegetation; (6) the distribution of the two species on the midday humidity gradient in forest matches their distribution on the geographic gradient of rainfall in the island.

(3) The problem is raised as to how mosquito densities become reduced in drier geographic areas. An interpretation is developed in terms of the microclimatic control of the mosquitoes' access to the bromeliad flora where they breed. vector population, and the density and species composition of this is regulated by the plantation microclimate.

The fact that the access of the mosquito to the village is conditioned in this way by the microclimate of the surrounding vegetation is at the basis of the geographic variation of vectors, the limitation of the problem as a whole to areas of high rainfall, and the correlation between malaria rates and precipitation across the center of the island (fig. 10).

The relationships of the forest, plantation, and vector populations of *Kerteszia* in the major climatic areas of Trinidad may be summarized schematically as follows.



Appendix

The Ecological Basis of the Vector Status of the Two Anophelines, and the Geographic Distribution of Malaria Rates

For the epidemiologist the focal point of a malaria problem is the "traffic in gametocytes." For the ecologist, interest therefore centers on those factors, human habits, and insect requirements, which lead to and regulate the degree of contact between man and the anopheline vector. In the Trinidad area to which *Kerteszia* transmitted malaria is restricted, the villages are always more closely associated with cacao plantation than with forest, and it is from the plantation that the evening flight of *Kerteszia* into the villages originates. Thus the cacao plantation *Kerteszia* population is the potential

The two entries enclosed in brackets, the canopy population in the extremely wet and the extremely dry areas, are inferred. The inference is based, as discussed earlier, on the established conditions in the cacao plantations in these areas and then by analogy from the known relation between cacao and canopy in the C zone.

In the wettest B areas humidities are high enough to permit *homunculus* to enter the fully illuminated plantations where large bromeliads are abundant and to bite man in and around the houses in the plantations. In the drier C, D, and E zones *homunculus* retreats out of the plantations and down the forest profile; it no longer transmits malaria as this distribution adjustment to its microclimatic demands divorces it from the human population in the village. Here, however, *bellator* is abundant in the forest canopy and

in plantations, and is the malaria vector. It does, as we have seen, become less abundant, however, along the C-D-E gradient as falling humidity renders the plantation breeding ground less accessible. Falling malaria rates parallel these declining *bellator* densities from C to E. Finally in the F zones where *bellator* can still be caught in the lower forest levels it has retreated (as *homunculus* did earlier) out of the cacao altogether; and here, with all other factors as before—houses in plantation, a large potential breeding ground in the plantation, and *Kerteszia* in the forest—transmission ceases.

It is interesting to recall here the basis of Knab's criticism (1912) of the original suggestion made by Lutz in 1903 that a bromeliad-breeding anopheline was the vector in the malaria epidemic he investigated in São Paulo, Brazil. Knab maintained that a forest mosquito like *Anopheles lutzii* (*cruzii*) would not bite man frequently enough to support parasite

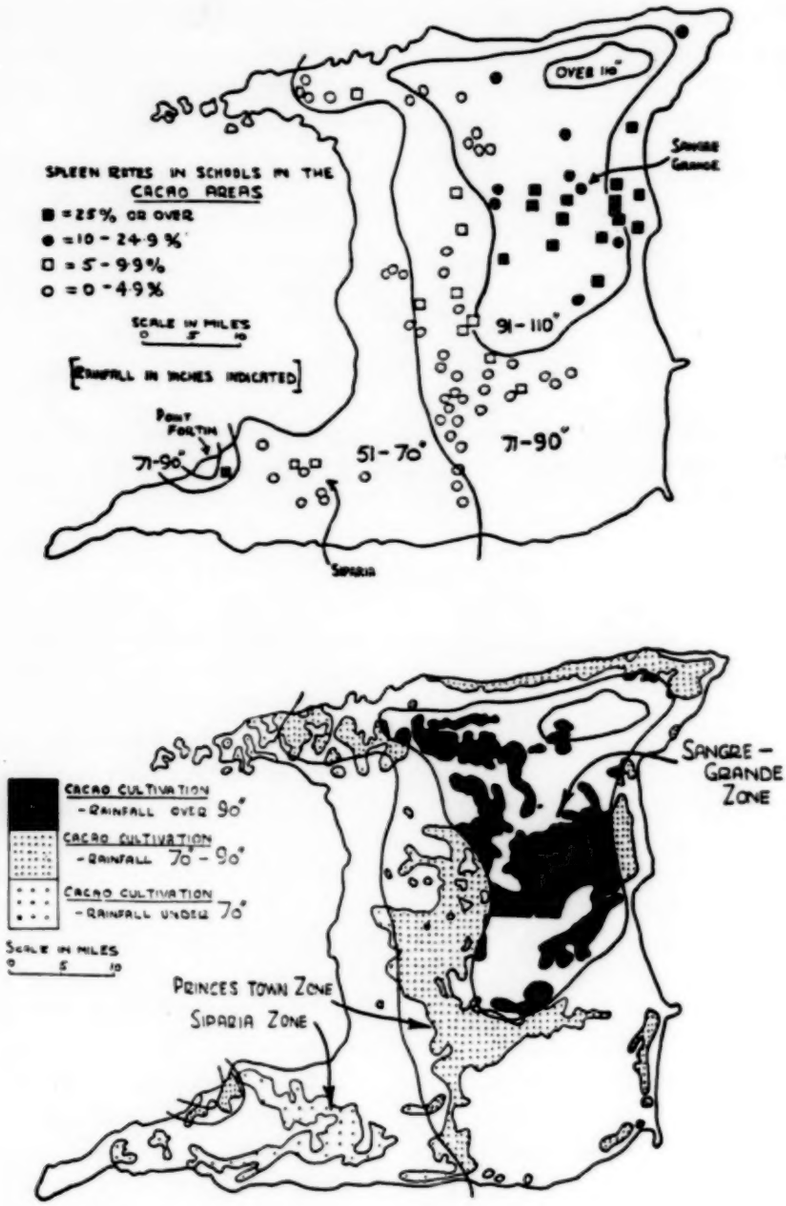


FIG. 10. The distribution of spleen rates (malaria due to *A. bellator*) and cacao plantation in rainfall zones. After Downs and Pittendrigh, 1946.

transmission. Knab does not make a clear statement of his reasoning, but evidently he felt that a forest mosquito would not be able to leave its forest environment to bite the road-builders around their open camps. Such an assumption was clearly made without knowledge of the ecological diversity of forest environments and faunas. The lower forest levels are remarkable and specialized environments with regard to their climatic stability, high humidity, and low temperature (Allee, 1926; Bates, 1945; Haddow, 1945), but from these specialized conditions near the forest floor there is, vertically, a gradient of internal climate which culminates in wholly unspecialized conditions in the extreme canopy.

The climate in the canopy is subject to all the daily and seasonal fluctuations of that in open country. Mosquitoes that have evolved in relation to the conditions in the forest floor zone are apparently restricted to the forest, since their microclimatic demands are not commonly met outside, but such a restriction is obviously not to be expected of species whose normal forest niche is in the higher, climatically unspecialized, canopy zone.

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THE ECOTOPIC SPECIALIZATION OF *ANOPHELES HOMUNCULUS;* AND ITS RELATION TO COMPETITION WITH *A. BELLATOR* [1, 2]

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INTRODUCTION AND THEORETICAL CONSIDERATIONS

The previous paper in this series (Pittendrigh, 1950b) began the discussion of the ecological divergence of *Anopheles bellator* and *A. homunculus* by listing five questions in relation to which the work was oriented. The data that were presented there bore on the first two questions raised, viz: the extent to which the geographic distributions were different, and whether these distributions could be attributed to adaptive differences between the species.

The results demonstrated that the species do diverge in microclimatic requirement in such a way that they exhibit markedly dissimilar distributions in the diverse humidity conditions that occur in the area where they are sympatric. Thus *homunculus,* which requires wetter conditions than its sister species *bellator,* occurs lower on the vertical gradient of humidity in forest; and its feeding activity is more concentrated in the moister periods of the day.

It was shown how this divergence in humidity requirement, through its effect on the access the mosquitoes have to the bromeliads where they breed, could account for the established discrepancy in geographic distributions. The epiphytic bromeliad flora, because it requires high light intensities, is always incidentally concentrated in the dry canopy niches of a given forest: the most fully illuminated niches are always the driest. If we compare conditions in two climatic zones, one drier than the other, it is clear that the humidity requirement of the mosquito can only be met in the lower and, therefore, darker levels of the forest in the drier zone. It follows that here access to the breeding ground will be diminished and population size correspondingly reduced. The geographic distributions of *A. bellator* and *A. homunculus* are interpretable in these terms. *A. homunculus* populations are at their maximum in the wettest part of Trinidad where this species has easy access to the entire bromeliad flora. They diminish in drier regions where *bellator* (whose lower humidity needs are attested by its higher distribution in forest here) becomes common. Finally the expansion of *bellator* into still drier areas ceases long before the bromeliad flora itself is materially diminished by decreased precipitation.

The effect of microclimate on the availability of the mosquito's breeding ground leads to interesting considerations in connection with the third question raised initially, viz: what are the ecological relations between the species in the area where they coexist? Are they out of competition with each other as theoretically is to be expected?

The range expansion of two closely related species, leading to a distribution overlap, raises problems of both ecological and evolutionary interest which have recently been discussed by Lack (1944, 1947, and 1949) and Crombie (1947). Lack points out that when the species meet they will compete and reach one of

[1] This paper is no. IV in the series "The Bromeliad-Anopheles-Malaria Complex in Trinidad."

[2] The studies on which this paper is based were made under the joint auspices of the Trinidad Government and the International Health Division of the Rockefeller Foundation.

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four more or less stable ecological equilibria, as follows: (1) complete elimination of one by the other; (2) the occupation of adjacent regions with no overlap; (3) the occupation of different habitats in a zone of overlap; (4) the occupation of the same habitats and regions but with the species eating different foods. The third and fourth alternatives could probably be fused and restated more generally as "occupation of the same general region but out of competition with respect to the limiting aspect of the environment whether this be food or available breeding opportunities." [4]

The recognition of these several possibilities and the significance of the problem as a whole derives from the work of Gause (1934, 1936) who has pointed out that two species which utilize precisely the same foods and are dependent in general on the same portion of the total environment cannot coexist without the extremely improbable further qualification that they be equally well adapted. Given any degree of selective advantage on the part of one species, the other must eventually be eliminated. This generalization is upheld by Gause's own experimental work on Protozoa (reviewed in Gause, 1936), and more recently by that of Crombie (1946) on the flour beetles of *Tribolium* and other genera. It lies at the root of the well-known statistical peculiarity of animal communities which Elton (1947) has recently discussed: viz., the extremely low number of species in one genus (on the average much less than two) present in one community (cf. Williams, 1947).

The case of *Anopheles bellator* and *A. homunculus* in Trinidad has several aspects that are remarkable in this context. First, the two species not only overlap in their distribution, but this overlap extends throughout the overwhelmingly greater part of *homunculus'* total range. This corresponds with the third general alternative, which has been compounded (above) out of Lack's third and fourth: there is overlap and this requires that the species be out of competition with respect to limiting aspects of their environment.

Problems immediately became evident if we review the breeding activity of the anophelines in the light of these theoretical considerations. We are led to expect that given fixed moisture requirements on the part of *homunculus,* this species could not invade to any significant extent, the drier territory occupied by *bellator.* Such an expansion by *homunculus* requires its retreat down the forest profile placing the species at a marked adaptive disadvantage in the exploitation of the available breeding facilities in the dry canopy (figs. 2 and 3).

So long as both species are equally dependent on the canopy flora, it is clear that they could not coexist. The most likely equilibrium position would be Lack's second alternative: the occupation of adjacent areas with no overlap, each species being adaptively superior on its respective side of the distribution boundary. Indeed, the fact that the two species do coexist so extensively is itself sufficient ground to infer that moisture requirement is not the only respect in which the two species diverge. A further differentiation must exist making *homunculus* independent of the dry canopy, and thereby obviating unequal competition with *bellator* for the same breeding facilities.

Experimental Results

(a) *Field observations*

Field observations on the relative frequency of *bellator* and *homunculus* larvae in different bromeliads initially indicated the existence of an ecotopic specialization on the part of *homunculus* that is significant in view of the foregoing discussion. Table 1 summarizes the field data concerned; the observations were all made in zone B and the wetter parts of

[4] There is at least one other possibility. That is as Lack (1944) notes where the two species are parasitized to such an extent that neither food nor breeding opportunity limits their population size, and in this sense they need not be "out of competition" for either factor.

TABLE 1. *Field observations on the differential utilization of large and small bromeliad species by* A. bellator (*b*) *and* A. homunculus (*h*)

Bromeliads	B zone			C zone		
	N	b	h	N	b	h
Large species						
Vriesia amazonica	0	0	0	37	480	7
Gravisia aquilega	63	12	5	184	501	15
Aechmea porteoides	43	0	14	0	0	0
Wittmackia lingulata	42	3	1	50	149	1
Small species						
Vriesia procera	58	0	83	43	32	9
Guzmania monostachia	690	0	214	45	11	2
Guzmania lingulata	298	0	99	0	0	0
Totals: large species	148	15	20	271	1130	23
Totals: small species	1046	0	396	88	43	11

N = number of plants, all of which were removed from the cacao trees or their immortel shade trees in plantations (cf. Pittendrigh, 1948). The extremely large number of small bromeliads examined in zone B does not reflect their true frequency relative to large species. The very low density of *bellator* in zone B made it necessary to examine this large sample to determine whether the utilisation of small species by *bellator* was significantly different from that of *homunculus* in this zone.

zone C. It is seen that if we segregate the recovered larval population from the lumped small bromeliad species and compare it with that from the lumped large species, the distributions of the two mosquito species differ strikingly: the total recovered *homunculus* population is far more heavily weighted into the small bromeliads than is the *bellator* population.

It is unnecessary here to discuss the likelihood that these results are due merely to coincidence of distribution between *homunculus* and small bromeliads rather than to any discriminating action or differential survival on the part of the mosquitoes: coincidence of distribution is known to be involved to some extent (Pittendrigh, 1950b) but the experiments given below demonstrate that either discriminating action or differential survival on the part of the insects is also involved.

(b) *Experimental breeding plots involving nine species*

Table 2 gives the data obtained from the large experimental breeding plots set up previously to determine the relative importance of the common bromeliads as *bellator* breeding places. The experiments, described fully elsewhere (Pittendrigh, 1950a) consisted of offering for oviposition replicated blocks of nine bromeliad species in a compact area on the ground. Since *homunculus* is common at ground level and oviposited in the experimental plots we may compare the utilization of different plants by the two mosquitoes. The extent of utilization of each bromeliad species is expressed by its breeding index, which is calculated from the experimental results as follows: total larvae per plant (of the tested bromeliad species)/total larvae in *Gravisia aquilega* (cf. Pittendrigh, 1950a).

It is clear that the breeding indices for the small *Vriesia* species (*V. procera*), and especially for the small *Guzmania* species are much higher for *homunculus* than for *bellator* (ratios are given in bold type in table 2). The indices for *Guzmania monostachia* were .03 for *bellator*, and .39 for *homunculus* in the first series; .08 *bellator* and .82 *homunculus* in the second series; and .05 *bellator* and .24 *homunculus* in the third. The *Guzmania*

lingulata values were .02 *bellator* and .61 *homunculus* in the second series, and .03 *bellator* and .17 *homunculus* in the third. *G. lingulata* was not tested in the first series. The differences between *bellator* and *homunculus* are very significant ($P < 0.01$) for two of the individual small species (*V. procera* and *G. monostachia*), and in all three series when the combined large bromeliads are compared (using chi-square) with the combined small species.

The microclimatic access of the mosquitoes to the large and small bromeliad species is the same in these plots. It is to be concluded, therefore, that the difference in the proportion of *homunculus* and *bellator* larvae in the large species such as *Gravisia aquilega* and in the small ones such as *Vriesia procera* and the *Guzmania* species is due to the greater ability of *homunculus* to utilize these smaller species.

(c) *Gravisia aquilega* vs. *Guzmania lingulata*

Further experiments were run to test this conclusion more rigorously than is possible with the few (3) replications offered by the plots already described. For this purpose the large bromeliad *Gravisia aquilega* (cf. fig. 4 in Pittendrigh, 1948) was extensively compared with *Guzmania lingulata*, which is the most abundant small species of the lower forest levels. In all, 39 plot results are available, comprising 1030 plants of each species. Table 3 gives an analysis of four plots that

TABLE 2. *The differential utilization of large and small bromeliad species by* A. bellator (*b*) *and* A. homunculus (*h*) *in the three series of large experimental breeding plots*

	homunculus indices in series			Mean breeding index		h_i/b_i	Significance of difference between b_i and h_i
	1	2	3	h_i	b_i		
Large species							
Vriesia amazonica		3.07	0.94	2.01±1.1	2.49±.12	**0.8**	P.4 <.5
Gravisia aquilega	1.00	1.00	1.00	1.00	1.00	**1.0**	
Hohenbergia stellata	.79	1.85	.70	1.11± .37	.59±.14	**1.9**	P.3 <.4
Wittmackia lingulata	.89	.71	.59	.73± .09	.45±.09	**1.6**	P.02<.05
Small species							
Vriesia procera	.59	.32	.27	.39± .10	.14±.04	**2.8**	P <.01
Guzmania monostachia	.39	.82	.24	.48± .17	.05±.01	**9.6**	P <.01
Guzmania lingulata		.61	.17	.39± .22	.03±.01	**13.0**	P.2 <.3
Aechmea nudicaulis	.11	.61	.06	.26± .18	.06±.06	**4.3**	P.3 <.4
Tillandsia fasciculata		.29	.00	.15± .15	.02±.02	**7.5**	

Total larvae in	Series 1		Series 2		Series 3	
	b	h	b	h	b	h
All large species	178	150	215	186	259	213
All small species	14	61	20	74	18	49
χ^2	31.0		32.4		18.4	
P	<0.01		<0.01		<0.01	

The breeding indices for *homunculus* (h_i) were obtained simultaneously with those for *bellator* (b_i) which have been discussed fully in an earlier paper (Pittendrigh, 1950a). The significance of differences between the mosquitoes' breeding indices is tested with $t = \bar{x}/s_{\bar{x}}$, where x is the difference between the species in each of the three experiments which gave paired observations for the mosquitoes in each plant species.

Total larval populations in large species (lumped) and small species (lumped) are tested with chi-square.

TABLE 3. *The interspecific difference between* A. bellator *and* A. homunculus *in their utilization of* Gravisia aquilega (*Grav*) *and* Guzmania lingulata (*Guzl*)

Date	Breeding plot	Total larvae per plot				Breeding ground indices for *Guzmania*	Significance	
		bellator		*homunculus*				
		Grav	Guzl	Grav	Guzl	$b_i < h_i$	χ^2	d.f. P
May '45	a	103	0	124	11	.00 < .09	7.05	1 < 0.01
	b	186	2	352	14	.01 < .04	0.25	1 > 0.05
	c	95	7	379	67	.07 < .18	4.73	1 < 0.05
	d	86	0	343	21	.00 < .16	3.99	1 < 0.05
June '45	a	97	2	128	24	.02 < .19	10.80	1 < 0.01
	b	125	4	500	58	.03 < .12	6.79	1 < 0.01
	c	114	5	516	112	.05 < .22	14.06	1 < 0.01
	d	84	0	538	54	.00 < .10	8.78	1 < 0.01
July '45	a	29	8	15	18	.28 < 1.20	8.10	1 < 0.01
	b	83	1	200	53	.01 < .27	16.86	1 < 0.01
	c	56	1	184	37	.02 < .20	7.40	1 < 0.01
	d	47	1	244	42	.02 < .17	4.75	1 < 0.05
Total χ^2							93.55	12 < 0.001
Total larvae		1105	31	3523	511	.03 < .15		

b_i and h_i are the *bellator* and *homunculus* breeding indices respectively for *Guzmania lingulata*. These are calculated as total larvae in *Guzmania*/total larvae in *Gravisia*.

were run on three consecutive months, giving twelve of the 39 results. *A. homunculus* made far greater use of the smaller species than *bellator;* only 3 per cent of the *bellator* larvae were found in *Guzmania lingulata* as compared with 13 per cent of the *homunculus* population. For all but one of the twelve separate samples chi-square shows the difference between the two distributions to be significant; the cumulative chi-square is overwhelmingly significant.

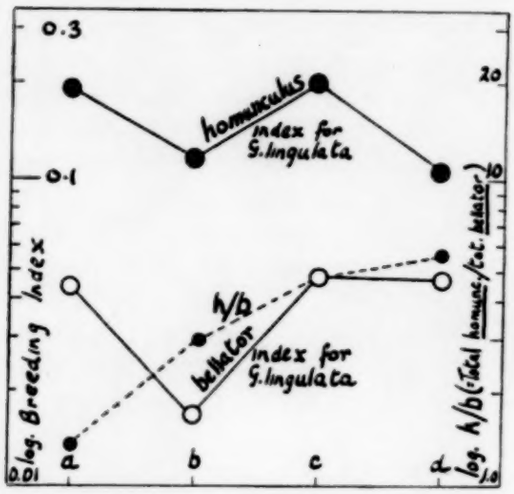

FIG. 1. Mean *homunculus* and *bellator* breeding indices for *Guzmania lingulata* in four progressively wetter breeding plots. See text.

a is the driest plot, and d the wettest. Data from table 3.

Breeding occurred in *Guzmania lingulata* in 22 out of the remaining 27 trials; and in 19 of these 22 experiments the *homunculus* breeding index for *Guzmania* (larvae in *Guzmania*/larvae in *Gravisia*) was greater than that of *bellator*. In all, therefore, the *homunculus* breeding index for *Guzmania* exceeded *bellator's* in 31 out of 34 trials ($P < 0.01$).

(d) *Gravisia aquilega* vs. *Vriesia procera*

The same result has been obtained from breeding plots containing *Gravisia aquilega* and the small *Vriesia* species, *V. procera* (cf. fig. 4 in Pittendrigh, 1948) : compared with *bellator, homunculus* made greater use of the small bromeliad species in 10 out of the 12 trials that were made.

(e) *Experimental reversal of the interspecific ratio in "an environment" composed exclusively of small plants*

Data from the B and C platforms in July 1945 provide an extremely striking demonstration of the difference between the two anophelines in their ability to utilize small bromeliads. In all previous platform samplings when a mixture of small and large plants (e.g., *Guzmania lingulata* or *Vriesia procera* and *Gravisia aquilega*) had been used, the total larval samples gave h/b fractions well below unity. Such indices are in agreement with all the adult data: that is, considering the forest profile as a whole, *homunculus* is definitely the less common species (table 4; cf. tables 5 and 8 in Pittendrigh, 1950b). In July 1945, the small *Vriesia* species, *V. procera,* alone constituted the breeding plots on the platforms; no large species were used at all. The fractions *homunculus/bellator* obtained from this sampling were above unity in both the forest (h/b = 1.09) and the cacao (h/b = 2.30). Clearly, this complete reversal of the numerical superiority of the species is due to the selectiveness of the sampling procedure. The apparent dominance of *homunculus* is an effect of its greater ability to utilize the plants offered.

TABLE 4. *Interspecific ratios* (homunculus (*h*)/bellator (*b*))

	Adult data*		Larvae data*	
	N	h/b	N	h/b
A.				
A platforms	2	0.27±.07	3	0.12±0.05
B platforms	5	0.31±.03	3	0.57± .01
C platforms	4	0.23±.03	1	0.32
B. Larval samples based on the use of the small† species				
Vriesia procera alone		B platforms		1.09
		C platforms		2.30

* A. All adult data; and all larval data obtained from breeding plots that included large bromeliads.

† B. Larval data from breeding plots that contained only the small species *Vriesia procera.*

(f) *The accentuated depression of the vertical distribution of* homunculus *while ovipositing*

A comparison of the vertical ranges of breeding and feeding activity in the two species brings out a further point of interest. Table 5A lists, for the B platforms, all the available breeding data and all the biting adult data except those of capture series 4 and 7, which sampled only 5 feet, 45 feet and 75 feet and cannot therefore be usefully compared with the larval data.[5] It is evident that the interspecific difference in vertical distribution is much more strongly marked in the breeding than it is in the feeding phase of the mosquitoes' activity. Moreover this increased difference is not due to any significant upward shift on the part of *bellator:* it is caused by *homunculus* shifting still lower down the forest profile when ovipositing.

(g) *Summary and conclusions*

The experimental results just described establish that in some way *homunculus* is adaptively superior to *bellator* in the utilization of small bromeliads as breeding places. The competitive inferiority of *bellator* in environments composed entirely of small plants is well attested by the complete reversal of the interspecific ration (h/b) in samples recovered from the forest platforms when only small plants were used.

No critical evidence is available to distinguish between two possible mechanisms by which the observed results could have

[5] The discrepancy between feeding and breeding ranges discussed in this paragraph implies that when interspecific ratios (*homunculus/bellator*) are calculated from ground level samples, the values will be higher when they are based on breeding plot data than when they are derived from captures of feeding adults. Table 5B lists h/b values obtained at 5 feet at the B and C platforms. Capture series nos. 4 and 7, which could not be used in the first part of the table (5A), conform with the other series in having h/b < 1.0 while all the larval data give h/b considerably exceeding unity. The C platform data are similar.

TABLE 5. *The lower distribution on the profile of forest shown by* homunculus *in its breeding activity (Br) as compared with its feeding activity (F)*

A. Activity below 25 feet as per cent total activity (B platforms)

Feeding (F)			Breeding (Br)			h-b	
Series	b	h	Series	b	h	F	Br
CS6	46	56	Mar. '45	33	85	10	52
CS6a	49	54	Apr. '45	59	84	5	25
CS9	47	60	Jun. '45	41	82	13	41

B. Interspecific ratio (h/b) at 5 feet (B and C platforms)

B platforms				C platforms			
Series		h/b		Series		h/b	
F	Br	F	Br	F	Br	F	Br
CS4	Mar. '45	0.28	4.7	CS5	Apr. '45	0.24	1.2
CS6	Apr. '45	0.70	2.0	CS6	Jun. '45	0.30	(14.4)
CS6a	Jun. '45	0.78	(6.9)	CS6a		0.43	
CS7		0.38		CS7		0.26	
CS9		0.48					

arisen. These possibilities are: (1) that both mosquitoes oviposit in the small species to the same extent but there is in the small species a differential survival (selection) of *homunculus* larvae; (2) that *homunculus* oviposits more frequently in the smaller bromeliads than *bellator*. Such evidence as there is favors the second alternative. It seems improbable that a constant effect can be due to selection in larval stages when it is produced in the interfoliar tank of several distinct plants whose water differs markedly: the water in *Vriesia procera* has a high humus content and is always brown while in *Guzmania lingulata* the water is clear and relatively free of humus. In spite of these conditions, they are both utilized by *homunculus* more than by *bellator* when offered as an alternative against large plants: their only common physical attribute is their small size. Further, it should be noted that the effect is independent of the relative density of the two species. Figure 1 plots the mean *Guzmania* breeding index for both *bellator* and *homunculus* in the four breeding plots a, b, c, and d listed in table 3. These four plots were in successively wetter parts of the forest 200 yards apart: as expected the h/b fraction rises in the wetter plots but the fluctuation of the *Guzmania* breeding indices are not correlated with these changing mosquito densities. The fluctuations run parallel in the two species.

It seems probable to the writer that the physical attribute of the bromeliads which is significant in producing the interspecific difference between the mosquito species in their utilization of large and small plants is the size of water surface offered and the closeness of the leaf blades immediately above the surface. Thus all the bromeliad species which *homunculus* utilizes more successfully than *bellator* have very small water surfaces, and the interfoliar space above this which is available for the ovipositing female is very confined. On this view *bellator* is supposed to require less confinement and larger water surfaces (cf. Russell and Rao, 1942)

Irrespective of the mechanism involved, however, the significance of the result is

A— THE RELATION OF A. bellator AND A. homunculus TO Gravisia aquilega AND Guzmania lingulata IN NATURE
THE VERTICAL DISTRIBUTION OF OVIPOSITION ACTIVITY
bellator
homunculus
B — THE RELATIVELY GREATER UTILISATION OF Guzmania BY A. homunculus IN EXPERIMENTS IN WHICH BOTH MOSQUITOES HAD EQUAL ACCESS TO BOTH BROMELIADS
BREEDING INDEX
GRAVISIA AQUILEGA
GUZMANIA LINGULATA

FIG. 2. The vertical distribution of anopheline oviposition in forest; its relation to the distribution of the breeding ground; and the associated ecotopic specialization of *A. homunculus*.

Oviposition distribution diagramed by smoothed curves based on mean per cent values given in table 8 (B platforms) of previous paper (Pittendrigh, 1950b). Bromeliad data are for forest strip (excluding its atypical left end which overhangs the river) given in figure 7b of the first paper in this series (Pittendrigh, 1948). This strip was in the immediate vicinity of the B platforms which gave the oviposition data. Only those bromeliads that made a significant contribution to the "breeding ground" are included. Large and small circles distinguish large and small species. All the former (*Gravisia aquilega* (G), *Vriesia amazonica* (A), and *Hohenbergia stellata* (H)) are in the upper levels of the forest. The small black circles indicate *Guzmania lingulata* (and the few plants of the closely similar *Vriesia simplex*): of all the small plants which alone penetrate the lower forest levels, these are the only two supporting anopheline breeding except for *Vriesia longi-bracteata* which is rare in the Tamana area. The open small circles are *Guzmania monostachia* which is limited to the canopy.

Breeding indices for *Guzmania lingulata* are from the experiments listed in table 3.

evident when we consider: (1) that the shade tolerant bromeliads of the lower forest levels are all small species of which *Guzmania lingulata* is by far the commonest, accounting for about 90 per cent of all individual plants; and (2) this is the plant population to which *homunculus* is limited in those areas where it overlaps with *bellator*.

Clearly the advantage *homunculus* possesses in exploiting *Guzmania lingulata* is precisely the type of specialization that theoretical considerations argue is necessary for coexistence with its sister species. And, finally, the fact that *homunculus*' oviposition is restricted to still lower forest levels than its feeding distribution argues is necessary—in terms of humidity requirement—may well be associated with its adaptive superiority to *bellator* in the exploitation of the particular segregate of bromeliads which occurs in these levels (fig. 2).

THE ORIGIN OF ADAPTIVE DIVERGENCE

The data presented in this and the preceding paper have concerned two quite distinct respects in which *Anopheles bellator* and *A. homunculus* are differentiated ecologically: (1) in microclimatic requirement, and (2) in ability to utilize a particular segregate of bromeliads as breeding places (an ecotopic differentiation). The following discussion considers the two questions which remain out of the five initially raised in the preceding paper: How are the two ecological differentials related to one another; and what bearing do the results of these investigations have on the problem of the origin of the two species?

As far as *homunculus* in Trinidad is concerned, there is one sense in which the two ecological differentials are clearly related: this mosquito's ecotopic specialization is related in a compensatory way

to breeding problems inherent in its microclimatic specialization. Given its moisture requirement *homunculus* would evidently be excluded from some 90 per cent of its present total range in Trinidad if it lacked its ecotopic specialization.

Further inquiry into the relationship between the humidity demands of *homunculus* and its fitness to utilize small bromeliads leads to the question of speciation. It is unfortunately true that the greater part of the available information on the ecology of closely related species is equivocal when it comes to inferring modes of origin. Consider, for example, two sibling species which are kept out of competition in their overlap area by an adaptive divergence that is also responsible for their ultimate geographic replacement. This general case may be written formally as:

Replacement Area x(=RAx)	Overlap Area (=OA)	Replacement Area y(=RAy)	
X	X; Yap^1	Yap^1	case (1)

where X and Y are sibling species and ap^1 [6] designates those adaptive properties of Y that eliminate competition in OA and lead to the complete replacement of X in RAy. It is impossible with such data alone to discriminate between sympatric and allopatric interpretations as to the speciation and adaptive divergence of X and Y. Since ap^1 is adaptively meaningful in both OA and RAy, the overlap can equally well be viewed as either primary with geographic replacement a consequence; or it may be itself regarded as a secondary phenomena consequent on the acquisition of ap^1 in geographic isolation. The discrimination, when it is made, must rely on general theoretical considerations extraneous to the particular case. It is this type of situation on which Moore (1949) aptly comments "—evolutionary theory contributes more to the understanding of the data, than the data contribute to an understanding of evolution."

Mayr maintains in his (1947) review of ecological problems connected with speciation that all forms of the sympatric hypothesis are in the long run not only faced with theoretical difficulties but are apparently unnecessary. His view is, in effect, that since no two environments are ever exactly alike ecological differentiation will virtually always be involved in geographic isolation, and siblings will *secondarily* overlap when they have acquired adequate divergence in initial spatial isolation. There can be no question of the wide applicability of this view: in many cases overlap requirements will be fulfilled by ecological differentials evolved elsewhere. It constitutes a discrimination between the interpretations available for case (1) above, and has extraneous considerations entirely in its favor.

However, the ecological situation described in this series of papers fails to conform to the scheme in case (1) in a way which the writer considers significant. Using the same conventions as in case (1) the *Kerteszia* situation in Trinidad may be written as:

RAx	OA	RAy	
X	X; Yap^1 ap^2	Yap^1 (ap^2)	case (2)

where X = *bellator*, Y = *homunculus*, ap^1 the microclimatic specialization of *homunculus*, and ap^2 the quite distinct ecotopic specialization. RAx corresponds with zone D, OA with C, and RAy with zone B in Trinidad. The two respects in which this situation differs from the general case (1) above form the crux of this discussion. They are as follows: (a) the fact that ap^1 (the microclimatic differential) could not of itself maintain the coexistence of the two species in OA; it is the adaptive superiority of *homunculus* in the utilization of small species of bromeliads (ap^2) which renders this possible. (b) Further, the adaptive significance of this ecotopic specialization of *homunculus* is not only obvious under

[6] In this and following formal statements adaptive properties (ap^1, ap^2) are written, for simplicity, for only one of the pair of siblings. If Y has adaptive properties (ap^1) that so differentiate it from X that competition between them is eliminated then clearly, by definition, X has such properties that differentiate it from Y.

overlap conditions, it (the significance) is limited to them. This is indicated in the formal statement by the inclusion of ap[2] in parentheses under RAy. While *Guzmania lingulata* is commoner in zone B in Trinidad than in zones C and D, its abundance relative to large species is here no greater than is that of the other small species *Guzmania monostachia* and *Vriesia procera* relative to large plants in the drier zones C and D. In this way *homunculus* is no more dependent on *G. lingulata* in B than is *bellator* on *G. monostachia* and *V. procera* in D. In short, both anophelines in their respective replacement areas have complete access to the main bromeliad population of large species in the canopy, and it is only in their overlap where *homunculus* is forced to lower levels that conditions arise which lend significance to the ovipositional peculiarities of this species.

Now, the crucial point at stake in Mayr's (1947) view of the origin of those adaptive divergences that permit secondary overlap is the implicit assumption of an ultimate geographic segregation of the selection pressures necessary to produce the divergences involved: that is, a geographic segregation of the ecological conditions into which the siblings segregate in their sympatric area. As far as the ecotopic peculiarity of *homunculus* in Trinidad is concerned we can discern no such geographic segregation. Moreover figure 3 which summarizes the relationships under discussion holds so far as is known for the distribution of large and small bromeliad types not only in Trinidad but elsewhere. Geographic regions (like B in Trinidad) which would be humid enough for the complete predominance of *homunculus* do not selectively eliminate large bromeliads. The particular bromeliad species may change but large types still prevail in the canopy, and the lower *shade* levels are the only place where there is a general trend to small species. Certainly this is true of the forests which the writer has seen in Venezuela and Brazil, as well as in Trinidad. And in Santa Catarina, Brazil, even the shade levels do not eliminate large types as completely as they do in Trinidad, for several large

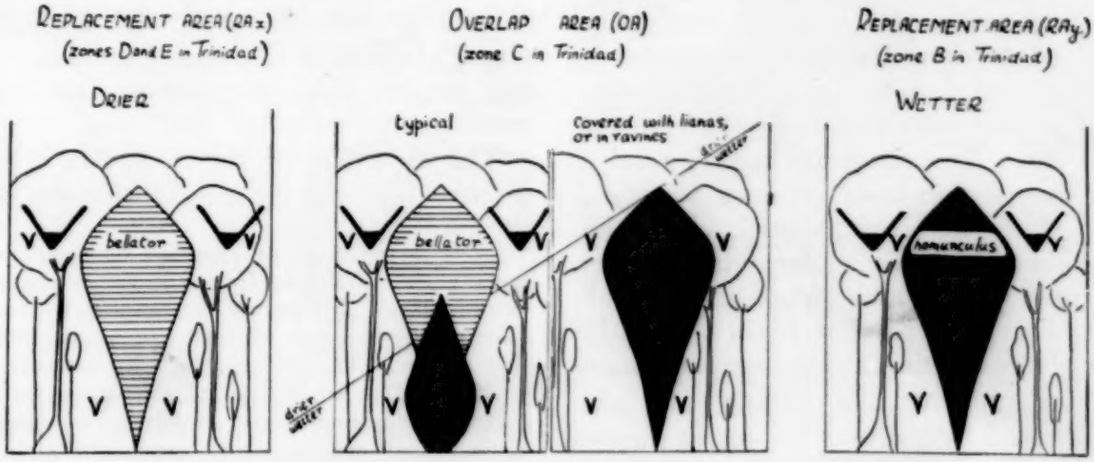

FIG. 3. Diagram to summarize the ecological relations between the anophelines and the bromeliad flora in three climatic regions.

Replacement area x is the driest and here *homunculus* is completely replaced by *bellator;* replacement area y is the wetter and here *bellator* is replaced by *homunculus;* the overlap area has an intermediate climate and *homunculus* is forced to lower levels to meet its humidity demands. In all regions large bromeliads occur in the canopy and are diagramed by large V's. Small bromeliads occur in the canopy and alone constitute the flora in lower levels; they are diagramed by small V's. In the overlap area the typical situation is indicated and also that which occurs in ravines or where lianas reduce light intensity and raise humidity to such an extent that large bromeliads disappear (cf. figure 7 in Pittendrigh, 1948) and *homunculus* locally dominates the anopheline population.

Canistrum species are here shade tolerant and occur alongside small *Vriesia* species in the shade levels filled in Trinidad almost exclusively by *Guzmania lingulata* and the other less common small species enumerated earlier. Thus, wherever it arose, the differential ability of *homunculus* to utilize small plants has evident significance only in relation to competition with another species in the canopy. If, therefore, we are to accept the view that the widespread ecological overlap between *bellator* and *homunculus* in Trinidad is wholly conditioned by differentials acquired in geographical isolation we have to invoke a completely fortuitous origin for the particular differential that is really responsible for their coexistence inasmuch as there is no evidence that *homunculus* would be under any greater selection pressure in its replacement area to develop its ecotopic specialization than *bellator* would have been to develop precisely the same ability in its own replacement area.

While these considerations still do not necessarily lead to any unique alternative interpretation, they have suggested to the writer that the ovipositional habits of *homunculus* have in all probability evolved under overlap conditions either in Trinidad or elsewhere as a result of selection pressures arising from the competition they (the oviposition habits) resolve. This suggestion leads first to a consideration of the sympatric spatial segregation hypothesis. This interpretation (cf. Mayr, 1949) would suppose that within a continuous population in an area analagous to zone C in Trinidad two sub-populations (*bellator* and *homunculus*) differentiated through spatial segregation in relation to drier and wetter parts of the forest, that is, in relation to both the vertical gradient of humidity and the mosaic distribution of excessively moist patches of forest such as those heavily covered with lianas or in ravines. In either case the humidity differential *within* such a climatic zone is matched by a change in flora because here increasing humidity goes hand in hand with decreasing light and leads to the elimination of all but small shade-tolerant plants. The merit of this interpretation is that it offers a determinate, selectionist, origin for both the microclimatic and the ecotopic specialization of the *homunculus* segregate; and to this extent it offers an immediate explanation of the presence of the ecotopic character in the secondary replacement area of *homunculus* where the character has no special adaptive meaning.

However, general theoretical considerations weigh heavily against the acceptance of spatial segregation hypotheses in general. The greater part of Mayr's (1947) criticism of sympatric speciation is not concerned with spatial segregation, but he notes the one important difficulty facing this hypothesis: it requires that considerable gene flow can be offset by selection. Obviously the question is not merely whether selection could ever offset gene exchange; it could were it strong enough and gene exchange weak enough. The problem is whether it could do so in face of the very considerable exchange that would occur between segregates such as *bellator* and *homunculus* on the vertical climatic gradient in forest, and the horizontal gradient in and out of ravines. In the present case this seems very improbable.

The rejection of the sympatric [7] hypothesis does not force us to abandon the

[7] The terminology of isolation processes has become somewhat complicated in the recent extension of the concept of geographic isolation (allopatric) to cover all forms of spatial isolation (Mayr, 1948, 1949; Lack, 1949 footnote to p. 301). The implication of this seems to be that only those processes involving no spatial displacement of population centers should be termed "sympatric." This serves to emphasize the diversity of putative mechanisms previously grouped under the term sympatric speciation, but it now introduces diversity into the processes involving spatial displacement of the diverging populations. It should be recognized that in considering the origin of adaptive divergence between siblings, the distinction between complete spatial *isolation* (geographic or allopatric in the old sense) and incomplete spatial *segregation* (cf. Mayr, 1949) is significant. This is well illustrated by the alternatives as

suggestion which led to its consideration. This was that the ecotopic specialization of *homunculus* had arisen in the overlap area because it is only here that we can envisage selection pressures of such a nature as to give rise to the interspecific differential concerned. An alternative explanation is available. We owe to Lack (1947, 1949) the explicit elaboration of the possibility that much adaptive divergence may develop between siblings in their overlap area subsequent to allopatric speciation. The distinction must be made in interspecific relations between mechanisms effecting reproductive isolation and those reducing competition (Lack, 1947; Mayr, 1949). The theoretical requirement of effectively complete spatial isolation for the attainment of reproductive isolation does not require the assumption that all adaptive divergence observable subsequently in overlap areas also evolved in spatial isolation.

Clearly some divergence acquired allopatrically is essential for any overlap to develop initially, but once this is established it may become more extensive as the new selection pressures that are precipitated lead either to the restrictive specialization of adaptive peculiarities already evolved elsewhere, or even to the origin of "new divergence" that will effect the reduction of mutual competition.[8]

Lack (1947) gives very clear examples of this process in birds. They may be written:

$$\frac{RAx}{X} \;\Big|\; \frac{OA}{X{:}Y\ ap'!} \;\Big|\; \frac{RAy}{Yap'} \qquad \text{case (3)}$$

Where the novel features are the detectable accentuation of divergence (ap'!) in the overlap area and the isolation of this from the replacement areas x and y as indicated by the vertical lines. Where the peripheral populations of allopatric species overlap there is far more likelihood that the consequent interspecific competition will lead to local evolutionary change if these peripheral populations be protected by isolation from the swamping effect of gene flow from the central (unaffected) populations.

The relevance of the process Lack has described to the problem raised in this discussion is obvious. Where part of the total adaptive divergence between two siblings has discernible relevance only to competitive conditions between them in their overlap we may well suspect that this divergence has originated subsequent to

they apply to the bromeliad-anophelines. The first hypothesis—(sympatric) spatial segregation—involves a selectionist account of the origin of the ecotopic specialization of *homunculus*. To this extent it constitutes a more attractive explanation of the presence of the ecotopic specialization in the replacement area (zone B) than the appeal to its completely fortuitous origin on which the second hypothesis—complete spatial isolation—must rely in this case. Although the hypothesis of spatial segregation is rejected for the present case, it should be stressed that incomplete segregation and total spatial isolation merge into one another and we have no reason at present to reject the possibility that speciation may occur between subpopulations sufficiently segregated to give selection the upper hand, but sufficiently in contact so that the selection *due to their competition* would give rise to some of the adaptive divergences necessary for their coexistence.

Also, since the whole discussion of sympatric speciation through spatial segregation devolves on the occurrence of sufficiently diverse intense selection pressures within a continuous array of populations, it is noteworthy that on *a priori* grounds, the necessary conditions are more likely to be fulfilled for some groups of organisms than for others. For example, it is clear that a given environment like rainforest is far more diverse in its potential selection pressures for a group like the insects than it is for the higher vertebrates which are largely independent of microclimate and in general far less exclusive in breeding ground and food requirements than the insects. If any concrete evidence is to be obtained indicating the efficacy of spatial segregation it is least likely to come from the higher vertebrates which perhaps significantly offer the bulk of concrete evidence in favor of current orthodoxy: total geographic isolation.

[8] Lack does not explicitly commit himself to the origin of "new" divergence in this way; his examples are all what may be termed "restrictive specialisation" of old divergences. But the origin of "new" divergence, I take to be implied in his emphasis on speciation and interspecific competition as major factors in the creative aspects of adaptive radiation.

speciation and as a consequence of selection precipitated by initial contact and partial overlap. In Lack's cases proof of this interpretation is available in the form of isolated populations in replacement areas where competition and the divergence concerned are absent. In the present case proof awaits an examination of large continental populations of *homunculus* that are out of competition with *bellator;* at present no such data are available.[9] Nevertheless of the three interpretations that have been discussed the secondary ecotopic specialization of *homunculus* is taken provisionally to be the most acceptable in view of all the facts. Indeed, it seems to the writer not unlikely that the particular character of *homunculus* we have been concerned with may prove limited to Trinidad where conditions for its development are remarkably favorable. All but a small fraction of the *homunculus* population is here subject to competition with *bellator.* Secondly, and this is the important prerequisite, it is isolated from populations out of competition with *bellator,* if indeed such exist. It is clear that the coexistence of *homunculus* with *bellator* could be achieved by the secondary development of diverse ecotopic differentials in *homunculus* in different places depending on the available opportunities to escape unequal competition with its sister species. In Trinidad *Guzmania lingulata* provides such an opportunity, and the special ability to exploit small plants is much more relevant here than it would be in Santa Catarina, for example (see above). The interpretation offered may be summarized as:

RA x Continental	Initial isolated contact and partial overlap in Trinidad; secondary overlap extensive following the development of ap^2			RA y Continental
X	RA x / X	OA / X; Yap^1 ap^2	RA y / Yap^1 (ap^2)	Yap^1

In concluding, attention is drawn to the situation Bates (1949) describes for *Wyeomyia* in Colombia. He notes that at least four species of this genus all occur in the Villavicencio forests where they are using quite distinct types of plant water containers (aroids, *Heliconia,* and *Ravanela* in this case) for breeding. While there is every likelihood that adaptive differentiation with respect to climate will always be involved in the allopatric speciation of insects, it is unlikely that such climatic differentiation alone will lead to more than very limited overlap. Even a forest offers only limited and graded climatic diversity and to this extent limited opportunity for the coexistence of competing (sibling) species. The high number of congeneric species that characterizes the mosquito fauna of many tropical regions itself argues for some divergence additional to ecoclimatic. And as a general case it seems unlikely that the intricate array of ecotopic differentiation that is called for, and is exemplified by Bates' case, corresponds with any geographically segregating conditions. In these groups secondary adaptive divergence that is precipitated by interspecific competition and exploits local opportunities to avoid it will in all probability prove widespread where the isolation prerequisite is met.

[9] The Trinidad populations of *bellator* and *homunculus* are peripheral fragments on the northeastern extremity of the range of both species, isolated by the severance of the island from the rest of the Paria Peninsular of Venezuela in Miocene or later times (Liddle, 1946). It is interesting that they have failed to cross the narrow water gap to Tobago in the north where an apparently ideal ecological situation exists for them in the mountains. *A. homunculus* is known from Colombia and Brazil and *bellator* from Venezuela, British Guiana, and Brazil. In a cursory survey of the situation in Sanata Catarina (Brazil) the writer found the two species showing the same pattern of climatic divergence that obtains in Trinidad: *bellator* occupies the relatively drier coastal area and is replaced by *homunculus* in the higher reaches of the wet Itajai Valley near Blumenau. But as yet nothing is known on the extent of their overlap if indeed there is any, or whether, if it exists, it is based on the same ecotopic divergence that exists in Trinidad. It is noted here that *bellator* does seem to overlap *cruzii* (a third *Kerteszia* species) extensively in the drier coastal area, and the ecological relations of these populations should yield interesting results.

The insistence on effective isolation as a condition for evolutionary change to ensue in local populations is the main point in Mayr's (1947) criticism of sympatric speciation. But in rejecting the latter he seems to criticize unnecessarily the inference that much of the adaptive divergence between siblings that is evident in overlap areas evolved locally because of its relevance to local problems. This inference, while it may suggest sympatric speciation does not require it. There is need for a careful examination of the precise nature of the ecology of siblings to determine how far they conform to the simple situation where one and the same adaptive divergence accounts both for the satisfactory elimination of competition in sympatric areas and for geographic replacement. And it has been the purpose of this discussion more to emphasize the possibility that local secondary divergence between sympatric siblings is widespread and ecologically detectable than particularly to urge acceptance of such an interpretation for the present case where proof is lacking but obtainable by studies on the South American mainland.

Summary

(1) The ecoclimatic differentiation of *Anopheles bellator* and *A. homunculus* is reviewed, and the conclusion is drawn that this divergence is not adequate to account for the extensive overlap which occurs in Trinidad. Some additional divergence is necessary to eliminate competition for the main breeding facilities provided by the forest canopy population of large bromeliad species.

(2) Field observations and experimental results are presented showing that *A. homunculus* possesses the additional ecotopic specialization which theoretical considerations argue is necessary for its coexistence with *bellator*. This specialization consists of the differential ability to utilize (oviposit in ?) the water provided by small species of bromeliads such as those that prevail in the lower forest levels.

(3) In discussing this result it is maintained that cases such as the present and that of *Wyeomyia* in Colombia offer difficulties for the hypothesis advanced by Mayr (1947) that adaptive divergence permitting the overlap of siblings is wholly of allopatric origin. The difficulty consists of the failure to detect any geographic segregation of selection pressures (ecological conditions) that would account for the interspecific divergence. Such a segregation is however immediately obvious in the area of sympatry. It is unnecessary to invoke sympatric speciation to maintain the position that some adaptive divergence, such as the ecotopic specialization of *homunculus,* has arisen in the sympatric area in relation to the problems inherent in interspecific competition. Such divergence may arise subsequent to allopatric speciation and secondary overlap, if the overlap area is isolated as it is in Trinidad.

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MODES OF EVOLUTION DISCERNIBLE IN THE TAXONOMY OF SNAKES

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The snakes compose the reptilian order Serpentes, which in most classifications is bracketted with the Sauria (the lizards) as a suborder of an Order Squamata. The relation to the lizards is especially clear from the remarkable copulatory apparatus of paired hemipenes, retractable by invagination into the base of the tail, which is peculiar to lizards and snakes. Morphologically the snakes represent a single family of lizards, much as the birds and mammals represent elaborated single orders of reptiles. The reason here proposed for establishing the snakes as a distinct order is an evolutionary one, somewhat corresponding to the evident reasons for regarding the derived birds and mammals as classes equivalent to the ancestral Class Reptilia. Renewed evolution, both in progressive lines and in adaptive radiation, has made the snakes fully equivalent, as a group, to the lizards, with a comparable number of families. The taxonomy of the Serpentes offers an intriguing subject for an evolutionary essay, because the group, while compact and definable, presents on the one hand clearly progressive evolutionary trends in the development of venom apparatus, and on the other many repeated adaptive radiations into and within the major habitats—the terrestrial, the burrowing, the arboreal, and the aquatic. The family classification employed requires surprisingly little modification from that of the Catalogue of Snakes of G. A. Boulenger (1896).

THE NORMAL SNAKE TYPE

If one draws in all of the aberrant families, the snakes may be difficult to define as a group, but the normal snake type is a very distinctive one. Great elongation of the body is accompanied by complete absence of the anterior limbs; great reduction or complete absence of the hind limbs; associated elongation of the internal organs, with reduction or loss of one of the lungs; a peculiarly efficient mode of locomotion by wriggling, with extreme flexibility of the vertebral column, aided by the distinctive character of transverse ventral plates, whose free posterior edges afford purchase against inequalities of the substratum. The most fundamental snake character is the capacity for swallowing whole food of a caliber greater than the body diameter of the snake. This is accomplished by the attainment of great flexibility of the jaw apparatus, by means of which the snake literally draws itself over the prey; and by extreme expansibility of the body, produced by the presence of soft skin between overlapping small dorsal scales. Once the evolutionary trend toward lightness and flexibility of the jaw apparatus had set in there could not be any development of the jaws or teeth for comminution of the food. A great many other general features of snake structure, such as the loss of the shoulder girdle, may be seen to be associated with the food-getting habits characteristic of the group.

The pattern of the head shields is strikingly fixed throughout the group, and may be regarded as a paleotelic inheritance from ancestral lizards. The modifications of this pattern, even in quite trivial details, may be directly associated with directions of adaptation and evolutionary changes of head form. The absence of an external ear and of a functional auditory apparatus is characteristic.

The eye, in which the clear "spectacle" evidently represents a fixed transparent

eyelid, exhibits remarkable differences from that of lizards and indeed from the eyes of all known vertebrates.

The Evolutionary Origin of the Snake Group

There are two opposing theories concerning the evolutionary origin of snakes, related to the two types of elongation of body, with reduction of limbs, found in lizards. Lizards, in several families, have become elongate and limbless in association with burrowing habits, with the development of a most distinctive habitus—consolidated head shields overlying a compact and reduced skull; vestigial eyes; ear openings absent; cylindrical elongate body, but with a short stubby tail; and uniform dorsal and ventral scutellation. This type and these characters resemble those of the two primitive snake families Typhlopidae and Leptotyphlopidae.

Another trend toward elongation in lizards, exemplified by the common "glass snake" (*Ophisaurus*), the blind worm (*Anguis*), the African *Chamaesaura*, and above all by the Australian family Pygopodidae, reaches quite the same degree of elongation of body and reduction of limbs, but with an elongate tapering tail, no reduction of skull or eyes, and sometimes at least without reduction of ventral plates. This lizard type points toward a central ancestral type of snake, from which the burrowing snakes with reduced eyes and shortened tails could very evidently be derived. Even the peculiar spectacle of the eyes of snakes has various analogues in lizards.

Gordon Walls, however, from the examination of the eyes of both lizards and snakes, comes to the very different conclusion that the primitive snake was an elongate lizard of the first type, in which the eye had become extremely degenerate. He accounts for the unique structure of the snake eye as an evolutionary development of structures *de novo*, as, in fact a parallel evolution of an eye from what, at least somatically, was no more than a vestigial retina. Walls has cogent arguments, but the whole matter requires renewed scrutiny; Walls' appears to have made the assumption that snakes with reduced eyes and a burrowing habitus (like *Typhlops*) are primitive, and this is quite untenable in any overall view of the snake group. Mahendra's phylogenetic scheme also assumes the Leptotyphlopidae and Typhlopidae to be the most primitive of snakes (Mahendra, 1938, p. 354, fig. 3). This seems to me entirely untenable; these families are the most specialized burrowers, their relations obscured by regressive evolution, though plainly derivable from a boid or proto-boid stock.

One evolutionary correlation of the rise and success of the snake type, with its abundant speciation and radiation into the major habitats and the available habitat niches, appears to be with the rise and speciation of the rodent group, which forms the basic food supply of the most abundant and successful stocks of snakes. Unfortunately, there is little information from paleontology that can be brought to bear on the problems of the origin and dispersal of snakes. The first known true snakes seem to be gigantic boa or python-like forms of the Cretaceous.

Levels of Progressive Evolution

Throughout the snake group there appears to be a trend toward development of a poisonous saliva and of correlated means of conducting it into the wounds of the animals preyed upon, and this direction of evolution is evidently progressive in the sense of increased efficiency of food getting. It should perhaps scarcely be necessary to point out that the use of the venom in defense against larger enemies is strictly secondary. A simple point of departure for the evolution of venom appears to be present in the mildly venomous reaction of the salivary secretions of even ordinary snakes, which, however, have no effect at all (except perhaps antibiotic) on the human blood.

The correlation of complete lack of venomous saliva, unmodified supra-temporal gland, and absence of apparatus for

injecting venom in the series of families of snakes in which vestiges of the pelvic girdle or of hind limbs are to be found establishes a primitive evolutionary level discernible in existing snake types, especially in the two families Boidae and Pythonidae. These snakes also have a considerable development of the right lung. We may think of the several specialized burrowing families with pelvic girdle vestiges as being derived from this level and from the generalized boa-python type.

A second level may be seen in the great majority of existing types of snakes of the family Colubridae, plus the specialized mud burrowers Xenopeltidae, the peculiar South Indian shield-tailed snakes Uropeltidae, and the extremely distinct aquatic family Achrochordidae. All of these snakes have the skull reduced to its final form by loss of the teeth on the premaxilla and loss of the coronary bone in the lower jaw, and all have the right lung reduced to a vestige. Most of the genera are without evident venom or venom conducting apparatus, but a large number, distinguished somewhat artificially as the subfamily Boiginae, have enlarged supratemporal glands and some of the teeth at the rear of the maxilla enlarged and provided each with a groove, evidently as a primitive and quite inefficient venom conducting apparatus. I shall return to the discussion of the subfamilies of the Colubridae in connection with adaptive radiation.

The third and most advanced level among existing snake types is that of forms with an extremely potent venom, in combination with an effective hypodermic apparatus for injecting it. This level appears to have been reached in two quite independent directions, by the development of hypodermic fangs at the front of the jaw (in the Proteroglypha); and by the development of an extremely shortened maxilla provided with only a single pair of hollow teeth (to allow for replacement, only one is ordinarily functional), and capable of rotation forward into effective striking position and of being folded back into the mouth when not in use (Solenoglypha). Each of these types has two sharply distinct families.

Clues as to the mode of evolution of the hollow fangs and of the folding fang are to be discerned by analogy in various biological series. The hollow needle-like fang is quite evidently formed from a fang with a groove on its anterior surface by the deepening and anterior closure of the groove—the resultant seam being readily visible in most proteroglyph fangs. Within the proteroglyph family Elapidae there is a clear trend from a primitive type with an elongate maxillary bone, with numerous grooved teeth in a series enlarging from rear to front, by shortening of the maxilla and reduction of the number of teeth to the single venom-conducting fang, with a paired socket, providing for growth and replacement. The perfected single-fanged type is seen in some cobras (*Naja*) and the American coral snakes (*Micrurus*). Surprisingly detailed evolutionary trends may be discerned in the fangs of snakes, as shown by Bogert's study of the venom-spitting apparatus of cobras (Bogert, 1943).

It is an interesting speculation, though perhaps only a speculation, to regard the fangs of the folding-fanged Viperidae and Crotalidae as derived from a rear-fanged snake ancestor by reduction of the maxilla from front to back, the opposite of the direction of reduction in the Proteroglypha. In any case, the rotation of the maxillary bone in both colubrine and boigine types may be highly developed; it functions to bring enlarged rear teeth into play (Boulenger, 1915; Haas, 1931). Mosauer's studies on the body myology of snakes (1935) and those of Haas (1931) on the jaw musculature have not yet been adequately integrated with other lines of evidence bearing upon the relations of the families of snakes. It seems evident that the field is open for effective further study, which is not attempted in the present essay.

Successive Adaptive Radiations

Even more interesting than the progressive trend toward the evolution of an effective venom that serves to bring relatively large prey under control are the repeated adaptive radiations, from each of the successive levels, into the major habitats—terrestrial, subterranean, arboreal, and aquatic. Review of the families and subfamilies of snakes brings out the fact that they fall into two sharply contrasting types—the generalized and the specialized. The generalized families are those that exhibit within themselves the capacity for adaptive radiation; the specialized are the *results* of one of the directions of such adaptation. These two contrasting types of category may be discerned in the subdivisions of any large group—the orders of reptiles, or of mammals, for example. For the most part the specialized families and subfamilies present aspects of irreversibility of evolution. All of the numerous biological series discernible among these several directions of adaptation appear clearly to be *directed* trends toward progressively improved adjustment to the habitat in question.

A further type of specialization, which, at least in the snake group, is associated in each case with only one of the major habitats, is that in the direction of a specialized food—as egg-eating, snake-eating, or eel-eating.

Among the families of the primitive level the only generalized types are the Boidae and Pythonidae. These families have variously been regarded as subfamilies or even merged as a single family. The touchstone of independent adaptive radiation, however, combined with radically different world distribution patterns, favors placing them as distinct families. The ovoviviparous habit, uniform in the Boidae, contrasts sharply with the oviparity of the Pythonidae; this character has not been given much weight, since in the higher snakes it is often clearly less sharply associated with taxonomic groups. It must be remembered that a "character" must be reexamined as to its weight in classification at every new evolutionary level, and indeed in every new group in which it is to be applied.

The specialized families at the level with pelvic vestiges are the two radically modified burrowing types, the Typhlopidae and Leptotyphlopidae, which represent the extremes of the burrowing habitus with cylindrical bodies, greatly modified head shields, vestigial eyes, short tails, and no ventral plates. Superficially they look extremely similar. In skull structure they are sharply different, and evidently represent two entirely independent entries into the subterranean realm. The third of the burrowing families, the Anilidae, is a mud or leaf-mold burrower, remarkable in having a genus in South America and two in Southern Asia.

The Typhlopidae (including the "Anomalepidae") and Leptotyphlopidae share with the Uropeltidae divergence from all other snakes in having two scale rows per body segment instead of one (Camp, 1923, p. 302). This phylogenetic lead seems not to have been followed out by subsequent studies. Mosauer (1935) has shown that the Typhlopidae are extremely specialized in body musculature.

In the intermediate level, the great majority of living types of snakes belong to the Colubridae, with its six subfamilies. Of these only the Colubrinae and Boiginae are generalized groups, but these include an almost bewildering series of terrestrial, burrowing, arboreal, and aquatic forms, each adaptational trend exhibiting numerous transitions, so that the inference of adaptational evolution becomes extremely convincing.

The fully distinct families in the colubrid level are the Uropeltidae, the shield-tailed burrowing snakes of southern India and Ceylon; the Xenopeltidae, a monotypic family whose representative has the habitus of a mud-burrower; and the Achrochordidae, a group of two genera, *Achrochordus* and *Chersydrus*, representing a most complete aquatic adaptation at the non-venomous level. These snakes

entirely lack ventral plates, have lost the usual snake head shields, and have the nostrils placed in raised eminences on the upper surface of the snout.

The specialized subfamilies of the Colubridae are the Sibynophinae, presumably snakes of peculiar food habits, essentially terrestrial or burrowing, with a peculiar structure of the lower jaw resembling that of *Xenopeltis;* the semiaquatic Xenoderminae, which point toward the Achrochordidae; the aquatic fish and frog eating Homalopsinae; the arboreal and slug eating Dipsadinae; and the terrestrial egg-eating Dasypeltinae.

The great remaining complex of the Colubrinae falls into two well-distinguished series in the Americas, placed by E. R. Dunn (1928) as the distinct subfamilies Natricinae and Ophiinae; these categories, tested against the Old World genera by Bogert (1940), do not seem to hold. The major remaining taxonomic problem at the family and subfamily level lies in this complex of about 250 genera, in which the Boiginae are to be regarded as evidently polyphyletic.

The Boiginae, sharply set off by Boulenger as the "Opisthoglypha"—the rear-fanged snakes—has been shown by numerous students of snake taxonomy to be an artificial assemblage of forms independently derived from specifically determinable "aglyph" genera. Thus *Chrysopelea* is derived from *Dendrophis* by Meise and Hennig (1935), who point to the development of a degree of venomousness in *Dendrophis,* which is without venom conducting teeth, and to the fact that the derived *Chrysopelea* has a varying number of grooved teeth at the rear of the jaw. This fact, together with the numerous grooved teeth of the primitive "proteroglyph" elapids seems to point conclusively to a tendency widespread in the Colubridae to develop grooves on the teeth, which obviously serve a venom conducting function.

At the third level, in which all of the snakes are characterized by an effective venom apparatus, on which they depend to subdue or obtain their prey, no less than three families are of the generalized type derived from stocks from the basic terrestrial habitat. The front-fanged Elapidae exhibit the subterranean or partly subterranean coral snakes, the arboreal mambas, the banded watersnakes of Africa, and a wide variety of terrestrial genera.

The folding-fanged vipers (Viperidae) are quite evidently more primitive than the pit-vipers (Crotalidae), also with folding-fangs, which have the highly elaborated specialized character of the sensory facial pit. Both Viperidae and Crotalidae clearly exhibit the capacity for adaptive radiation as may be seen from the following generic examples:

	Viperidae	Crotalidae
Terrestrial	*Vipera, Bitis*	*Crotalus, Lachesis*
Arboreal	*Atheris*	*Trimeresurus* (part)
Burrowing	*Atractaspis*	——
	Cerastes	*Crotalus* (part)
	[*Aspis*]	
Aquatic	——	*Agkistrodon* (part)

The adjustment to the burrowing and arboreal habitats has reached a fixed generic level in the Viperidae and not in the Crotalidae, which fits the supposition that the vipers are the older group.

Returning to the front-fanged group there remains the specialized family of the sea snakes, the Hydrophidae, with two subfamilies Laticaudinae and Hydrophinae. The Laticaudinae, though very aquatic and provided with a greatly flattened oar-like tail, are almost an ideal intermediate between the Hydrophinae and the patently ancestral Elapidae. The Hydrophinae exhibit every characteristic of the aquatic snake developed to an extreme—ventrals completely lost, nostrils superior, nonimbricate scales, greatly flattened tail—whereas the Laticaudinae have functional ventrals, overlapping scales, and more nearly normal head shields. The Laticaudinae, finally, lay eggs in crevices in coral above tide-mark, whereas the Hydrophinae are completely free from

the land and produce living young at sea.

The sea snakes are naturally fish eaters with a strong predilection for eels, curiously enough for the banded eels of tropical seas, which many of the sea snakes strikingly resemble.

Evolutionary Adaptation to Environment

Adaptive responses to mechanical needs, apparently focussed upon mechanical stimuli, are repeatedly to be found in the highly adapted forms. Arboreal snakes, for example, tend to great elongation of the body, except in the vipers and pit-vipers, in which a normal short-tailed stocky body form was the level of departure. Arboreal forms in these families accordingly develop a characteristic prehensile tail from which the little viperine *Atheris* or crotaline *Trimeresurus* hangs suspended, with the opportunity to form a horizontal coil in the air from which to strike.

In the arboreal colubrid snakes, elongation of the body is the rule, often with striking parallels in two quite distinct types of cryptic coloration. Two types of head form are combined with the elongate body—extremely elongate and greatly shortened. The elongate body may be seen to be capable of lateral compression or to be permanently compressed, and one needs only watch a living *Imantodes* bridge from one branch to another to see how effectively the I-beam principle comes into play. Indeed, this is carried to a great extreme in some snakes with greatly compressed bodies, in which the mid-dorsal scale row is enlarged to about the width of the ventral scales. Expansions of the dorsal spines of the vertebrae of certain snakes support such enlarged dor-

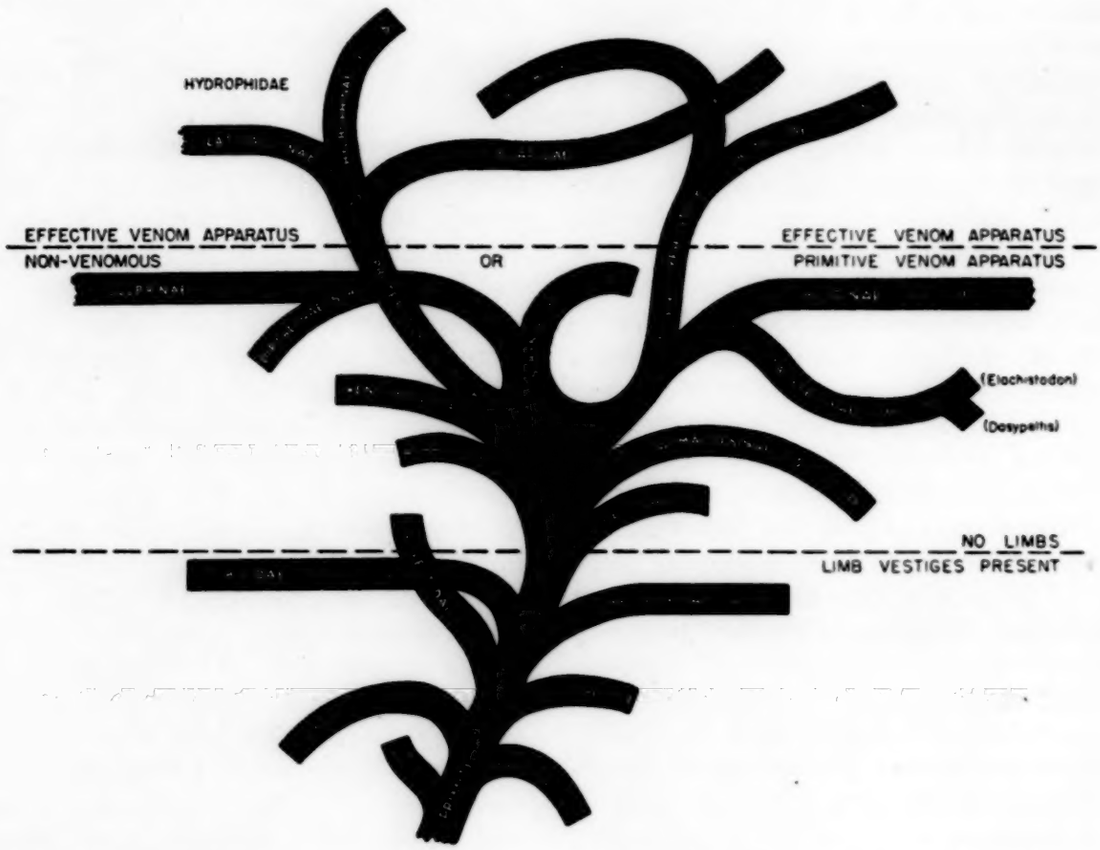

Fig. 1. Evolutionary diagram of the families and subfamilies of snakes. Generalized families indicated by (G); specialized by (S); approximate number of genera in each family indicated in arabic numerals.

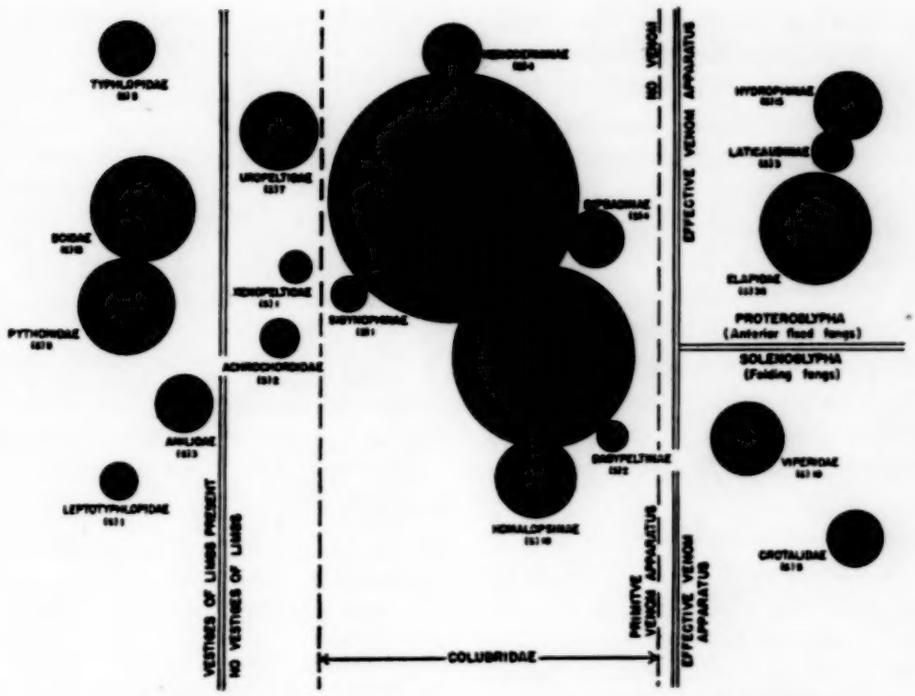

FIG. 2. Phylogenetic "family tree" of the families and subfamilies of snakes, in cross section representing the trunks and main branches. Symbols as in figure 1.

sal scales, but the functional relation is quite unknown in them.

Perhaps one of the most remarkable of the exact adjustments of snake structure to function is that of the egg-eating snakes, the Dasypeltinae, in which the esophagus is supplied with an "egg-saw" formed by the elongated and sharpened ventral spines of a series of neck vertebrae, the spines entering the dorsal wall of the esophagus. These snakes feed mainly upon eggs. The egg is swallowed to the level of the egg-saw, where a little pressure pierces the egg shell, the contents of which are forced into the stomach by the pressure of the distended neck; whereupon the pellet of egg shell is regurgitated. By one of the most recent improvements in snake classification the two genera, *Dasypeltis* in Africa and *Elachistodon* in India, in which this remarkable apparatus is found, are now associated in the subfamily Dasypeltinae. They had for years been widely separated because *Elachistodon* has grooved rear fangs and *Dasypeltis* has not. *Dasypeltis* however, has a greatly reduced dentition, and it is far simpler to infer the loss of the fangs than to suppose that so extreme an adaptation as the esophageal egg-saw had arisen independently (Malcolm Smith, 1943).

A notable adjustment in body form to the mechanical requirements for "striking" in a venomous snake is to be seen in the "plesiosaur form" of the more highly developed sea snakes. These creatures, especially in the genus *Hydrophis*, reach a large size (six to eight feet length) and have peculiar proportions, in which the head is almost ridiculously small and the anterior half of the body slender, its diameter no more than a third or even a fourth of that of the posterior third of the animal. Quite evidently the inertia of the bulky posterior part of the body makes it possible for the snake while freely suspended in the water to extend its neck in the rapid stroke required to reach its prey; and this of course is in part an explanation of the body form of the plesiosaurs and of the long necked turtles as well (Boulenger, 1913).

The highlights of interest in the classification of snakes are presented as a sketch of a more extended treatment, long planned. It appears to me that the classification fits extraordinarily well into an evolutionary framework. That the evolutionary patterns discerned are matters of inference must be freely admitted. Snakes have left few fossils, and their genetics is only beginning to be examined. The systematist must accordingly present his inferences to his colleagues in the hope that they may derive useful comparisons or clues for a more highly developed systematics of the future.

I have attempted to set forth the relations of the families, with an indication as to their number of genera, and as to whether they are specialized or generalized, in the usual type of so-called family tree (fig. 1). Such a diagram is so evidently misleading that I have redrawn it also in cross section, or rather in cross section of a tree with shorter and shorter branches on one side, like a wind-modified *Acacia* (fig. 2).

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PORCUPINES, PALEOGEOGRAPHY, AND PARALLELISM [1]

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INTRODUCTION

One of the vexing problems of geographic distribution is the explanation of why one suborder of rodents, the Hystricomorpha, is characteristic of South America, the southern parts of Eurasia, and Africa, but does not seem ever to have been present in North America until very recent times. Few authors have doubted that the Hystricomorpha represent a natural group with a common ancestry. In order to explain this distribution, it has been necessary to invoke continental drift, land bridges, trans-oceanic floating, or other hypotheses which seem rather dubious to many students. An attempt will be made to show that an alternative interpretation, involving only more orthodox and established migration routes, is at least as well supported by the available evidence.

The Hystricomorpha include a basic group of recent forms, together with their fossil relatives, which are almost universaly considered to be closely related. This group includes numerous South American rodents (porcupines, guinea pigs, chinchillas, agoutis, pacas, vizcachas, capybaras, etc.); our North American porcupine (*Erethizon*); the African, South Asiatic, and southern European porcupines (Hystricidae); and the African Petromyidae and Thryonomyidae. In addition, a number of other families, restricted to Africa, whose correct taxonomic position is very uncertain, are often placed here (Bathyergidae, Ctenodactylidae, Pedetidae and Anomaluridae).

[1] This study was assisted by a grant from the Marsh Fund of the National Academy of Science.

DISTRIBUTION OF THE HYSTRICOMORPHA

Since *Erethizon* is clearly of South American origin, having reached North America only after the union of the two continents at the close of the Tertiary, the centers of radiation of the modern Hystricomorpha are obviously South America on the one hand and Africa and perhaps southern Asia on the other.

During nearly all of Tertiary times, the hystricomorphs made up the entire rodent fauna of South America, the few non-hystricomorphs in the present and recently extinct faunas being mouse-like or squirrel-like forms, derived from North America, which moved into South America after the connection of the two continents through the isthmus of Panama in the late Tertiary. At the same time, a few South American hystricomorphs moved up into North America, of which the only survivor, north of Mexico, is *Erethizon*. At no time in the earlier Tertiary are there any known fossils from North America which can be interpreted as being hystricomorphs.[2] The only Tertiary North American rodents that show any indications of being related to the hystricomorphs are certain Eocene rodents, particularly the Paramyidae, which are sufficiently primitive and generalized so that they were very close to being ancestral to all the later rodents. But, by no stretch of the imagination could they be considered to have been hystrico-

[2] One form, *Hystricops*, was described by its author as a porcupine (Leidy, 1858). However, Stirton (1935, p. 414–415) in his review of the beavers, considers this to be only a somewhat aberrant beaver, an opinion concurred in by all other students.

morphs, since they belonged to a different and more primitive suborder.[3]

The South American hystricomorphs are all closely related, and, as we go back to the earlier fossils, they are continually less and less diverse, until, when we reach the earliest known South American deposits in which rodents have been found, the Lower Oligocene Deseado, there are only four or perhaps five families represented. There is sufficient structural unity among these early forms so that there seems to be very little doubt but that they are descended from a common ancestor which lived in the latter part of the Eocene. Therefore, only a single rodent type would need to have been brought into South America to permit the development of its hystricomorph fauna. Although no rodents are known from South America before the beginning of the Oligocene, our knowledge of the Eocene is essentialy limited to Patagonia, so that the exact time of their arrival in the rest of South America is uncertain. However, since the Eocene faunas of Patagonia are well known (Simpson, 1948), and since rodents are fairly numerous in Patagonia beginning with the Deseado, it seems reasonable to assume that they had only just reached Patagonia at that time. Although we do not know when they reached South America, it seems probable that it was not long before Deseadan time. A reasonable hypothesis would involve their having arrived in South America in very late Eocene time.

The true porcupines of the Old World, the Hystricidae, are sparsely represented in the Oligocene and later deposits of southern Europe, the late Tertiary of India and China, and the Pleistocene of Africa. Their rarity as fossils in the Tertiary of Europe and Asia suggests that Africa may have been their center of distribution. When they first appear, they are already true Hystricidae, with no indications of approaching closely to any ancestral type.

The other families of Old World hystricomorphs are of essentially unknown relationships. They may or may not be related to the Hystricidae or to each other. They are all practically unknown as fossils, though a few fragmentary specimens have been referred, often with doubt, to some of these families. Due presumably to the rarity of fossil mammals from Africa, we know nothing of their evolutionary history.

Explanations for the Distribution

Several possible explanations for these facts of distribution have been suggested. It has been proposed: (1) that the South American forms are descended from Old World (presumably African) rodents which crossed what is now the South Atlantic; (2) that the African forms are descended from South American ones; (3) that an Old World hystricomorph spread across Asia and North America into South America; or (4) that the two groups have been independently derived from non-hystricomorph ancestors. Some authors merely avoid the problem, and consider all the hystricomorphs to be related, without committing themselves as to the mechanics of distribution involved.

The first two alternatives would require continental drift, trans-oceanic land bridges, trans-oceanic island chains, or trans-oceanic floating of a pregnant early hystricomorph on a natural raft. The third and fourth possibilities require either arrival in South America before it was separated from North America, use of a Panamanian or Antillean island chain, or long distance raft floating.

The first hypothesis, that the South American hystricomorphs are descendants of rodents whcih crossed the South Atlantic, has had numerous supporters. Some of these have merely implied that this is what occurred (Scott, 1937, p. 262; Simpson, 1940c, p. 685; Wilson, 1949, p. 129), but others have been more

[3] Suborder Protrogomorpha according to Wood, 1947; Superfamily Aplodontoidea of Suborder Sciuromorpha according to Simpson, 1945 and Wilson, 1949. The difference is largely one of terminology, there being agreement as to the fundamental relationships of the forms.

explicit (Loomis, 1914, p. 184; Romer, 1945, p. 504).

Although Simpson has demonstrated, in connection with the Tertiary history of South America, that the evidence of mammalian paleontology is clearly opposed to drifting continents or trans-oceanic ponton bridges,[4] he leaves the question of the hystricomorphs open as perhaps being an exception to the general rule that the South American mammal fauna was derived from North American ancestors (1940a, p. 763–764; 1940c, p. 685; 1943a, p. 12).

From some time in the Paleocene until the Pliocene, all the fossils indicate that South America was isolated from the rest of the world and that the South American mammals were evolving in directions of their own, not influenced by immigrants from the rest of the world, with two or three exceptions, of which the hystricomorphs and perhaps the primates are the most striking (Simpson, 1943b, p. 417). This certainly means that there were no broad continental connections such as the drift hypothesis would involve, nor even a connection by a continuous land bridge, since, with interconnections of this sort, there must be an exchange of faunas and not merely of single forms.

The possibility of a chain of islands (either trans-Atlantic or via Antarctica) remains to be considered. This would permit a sweepstakes route to develop, under which the unilateral exchange of only a single member of the fauna is possible (Simpson, 1940b, p. 152–157). Although there is no geologic evidence that there was such a route during the Tertiary, this is not surprising since most of such a route would be beneath either the ocean or the Antarctic ice cap at the present time. Therefore, the hypothesis that such an island route was used must be further analyzed from the point of view of the relationships of the hystricomorphs themselves. This will be attempted below.

The possibility that a pregnant early ancestral hystricomorph floated across the South Atlantic from Africa to South America on a natural raft has been suggested by authors who consider that the South American forms have been derived from Old World ancestors, but who believe in the sanctity of continents (Matthew, 1915, p. 231; Wilson, 1949, p. 129). The floating hypothesis seems so improbable, in view of the great distances involved and the time and food supply that would be required for the trip, that it can almost be relegated to the category of the impossible, and, I believe, should be seriously considered only if every other explanation is shown to be impossible.

The second explanation for the known distribution, involving migration from South America to Africa, has been much less popular, probably because it still leaves the problem of the origin of the South American hystricomorphs unsolved. Joleaud (1919) has been nearly alone, since the days of Ameghino, in suggesting such a direction of migration. As indicated above, neither continental drift nor a land bridge could be used as the migration route, and an island chain or trans-Atlantic floating remain the only possibilities. Due to the directions of the currents and of the prevailing winds (at least at the present time), floating eastward seems even more unreasonable than floating from Africa to South America.

Since it appears almost certain that the ancestral hystricomorphs reached South America over an island chain, it would seem intrinsically more reasonable to suppose that this island chain connected with North America rather than with the Old World, as we know that islands were present, at least part of the time, along both the line of the West Indies and the Central American axis, and we do not know that they were present in any other direction.

[4] That his work has not been adequate to eliminate the ideas of drifting or of trans-oceanic land bridges is shown by the recent statement by Wolfson, that "it would seem that future biogeographical studies cannot avoid the use of the multiple geological hypotheses of permanence, drifting and foundering." (Wolfson, 1949, p. 1931.)

Transfer by accident, from one island to another, would readily explain the arrival of hystricomorphs in South America without other faunal interchange. It might be argued that, since other mammals did not use this route, the ancestral hystricomorphs probably did not. However, this is no more valid than is an argument that, since no other terrestrial placentals invaded Australia during the Tertiary by way of the East Indies, the Muridae did not. Actually, the South American monkeys may have gone from Middle America to South America at about the same time as the hystricomorphs or slightly later, along the same route (Stirton, 1949, p. 1922). If this were the method of arrival, the immigrant could either have been an Old World hystricomorph, which had spread across North America, or some native North American rodent which was developing hystricomorph tendencies independent of the Old World forms. These are the third and fourth of the possible explanations listed above.

The third hypothesis, that the hystricomorphs crossed North America leaving no trace of their transit, has probably been the most popular opinion of the conservative school, though few authors have specifically supported it in recent years. Since the hystricomorphs presumably were not differentiated before the Middle Eocene at the earliest, this represents the earliest date at which they could have crossed North America. This is after the breaking of the continuous land connection between the American continents.

When dealing with fossils, one must always remember that the absence of certain forms in any specified region merely indicates that such fossils have not yet been found there. This may be because they did not live there, or it may be due either to the accidents of collecting or to the absence of fossiliferous strata of the right age. However, the continental Tertiary deposits of western North America are among the most wide-spread of the world, they are abundantly fossiliferous, and they have been both extensively and intensively investigated for many years. Even twenty-five years ago, Matthew (1915, p. 230) considered it very doubtful that the absence of porcupines from the early Tertiary of North America was due to accidents of collecting, and, with the greatly increased collections now available, it seems almost incredible at the present time that such should be the case. The only conclusion that appears to be at all probable is that there were never any hystricomorphs in North America until the arrival of the few invading forms from South America at the close of the Tertiary, by which time they had already attained their modern form and evolutionary status.

The fourth hypothesis, that the South American group has been derived from non-hystricomorph North American ancestors independently of the Old World hystricomorphs, has never been extensively championed in print. Such a hypothesis, however, would solve all the problems of distribution, and would be much the most preferable if it were supported by morphologic or paleontologic evidence. As will be indicated below, there is some paleontologic evidence which suggests that this may be the correct explanation. A variant of this hypothesis, that the South American forms are descended from Paleocene immigrants from North America, has been suggested by Wilson (1949, p. 129), only to be discarded as too improbable for consideration.

Origin of the South American Hystricomorphs

Since the previous analysis did not settle the question of how the hystricomorphs reached South America, another approach will be tried. Can we solve the distributional problem by determining the group of rodents that actually were ancestral to the South American forms? Several groups of rodents have been suggested as being the possible ancestral stock. These include the Hystricidae and

Theridomyidae of the Old World and the Sciuravidae and Paramyidae of North America.[5]

The appearance of the hystricomorphs in the early Oligocene of South America indicates that, if they crossed from the Old World, it must have been shortly prior to that date. The Hystricidae are not known from deposits as early as this. Moreover, the early members of the Hystricidae are much too specialized, in the peculiar developments of their cheek teeth, to be ancestral to the Deseadan forms, nor is there any suggestion that any of their near ancestors could have given rise to the South American forms.

The Theridomyidae have often been considered to be ancestral to the hystricomorphs in general. The members of this family (excluding those forms that are frequently but almost certainly erroneously referred here) are known from the Upper Eocene and Oligocene of Europe. They show superficial similarities to the hystricomorphs, particularly in possessing a large infraorbital foramen, but they also show fundamental dissimilarities from the South American hystricomorphs at least, particularly in the tooth pattern. The most readily observed of these differences is the presence of five cross crests in the upper cheek teeth in *Theridomys*, instead of four as in the South American Hystricomorphs, the central one being the extra one. The differences are sufficiently marked so that it seems clear, on morphologic grounds, that the Theridomyidae cannot be ancestral to the South American forms (Friant, 1933, p. 1059; Wood, 1949, p. 46). Those few students who have recently studied the theridomyids apparently consider them to be a separate line, which had independently acquired an enlarged infraorbital foramen. No information is available to the present author to indicate whether the masseter actually penetrated the infraorbital foramen, as it does in the hystricomorphs. It is possible that the Theridomyidae may be ancestral to some or all of the Old World forms referred to the Hystricomorpha, but this can only be determined by detailed studies of the Old World fossil and recent hystricomorphs. Genera often referred to the Theridomyidae include *Phiomys* and *Metaphiomys* from the Oligocene of Egypt, as well as various Miocene African genera. These are just as different from the South American group as are the theridomyids, although they may fit into the ancestry of the African hystricomorphs.

Two North American families might include the ancestral stock, the Sciuravidae and the Paramyidae. The Sciuravidae show a number of important resemblances to the earliest South American hystricomorphs. Unfortunately, their skeletons are unknown, so comparisons must be limited to the characters of the skull and dentition. The teeth of some members of the family are closer to those of the South American hystricomorphs than are those of any other possible ancestor (fig. 1, A, B, D, E). In spite of the absence of supporting evidence from the skeleton, the South American group may have been derived from some unknown member of the Sciuravidae (Wood, 1949, p. 46–47).

The Paramyidae are abundant in the Eocene of North America. A few specimens are known from the European Eocene, suggesting that they had a Holarctic distribution. The best known members of the family are the Middle Eocene (Bridgerian) forms from Wyoming. These show no hystricomorph specializations, representing something not very far removed from the basic stock of all rodents, so it is not surprising that they show resemblances to the South Ameri-

[5] The Sciuravidae and Paramyidae are considered by many authors (Simpson, 1945, p. 200–201; Wilson, 1949, p. 87–88) to be subfamilies of the family Ischyromyidae, a group which the present author considers a superfamily. Authors who suggest the Ischyromyidae as the ancestors of the South American hystricomorphs are using the term in the sense of Simpson and Wilson, and presumably have in mind the animals here called Sciuravidae and Paramyidae.

can group. In particular, the Bridgerian *Reithroparamys* shows a number of features which suggest that it may have definite affinities with the South American genera (Wood, 1949, p. 46). Work now in progress on the Paramyidae (Wood, in mss.) shows that at least one genus of this family, *Rapamys* from the Upper Eocene, had enlarged the infraorbital foramen and had begun the "hystricomorph" type of masseteric specialization, in addition to showing cheek tooth modifications trending in the same direction as do the Sciuravids and South American forms (fig. 2). While there is a considerable structural gap between the Upper Eocene *Rapamys* of California and the Lower Oligocene *Platypittamys* of Patagonia, particularly in the upper molars, this is no more than would be expected given the geographic and temporal gap, and the Paramyidae may well have been directly ancestral to the South American hystricomorphs, through *Rapamys* or a closely related form. Upper Eocene rodents from Mexico and northern South America could well fill the structural gap.

It seems likely that the Old World hystricomorphs were derived from Old World members of the Paramyidae, either directly or through the Theridomyidae. They certainly could not have been derived from *Rapamys,* however, on the basis of what we know of the tooth anatomy of the various forms. Any detailed derivation, for the time being, must remain purely hypothetical, since the intermediate stages are unknown. Tentatively, it is perhaps most likely that the Old World hystricomorphs were derived directly from unknown (perhaps African) members of the Paramyidae.

One important problem remains which requires an explanation. The similarities between the African Petromyidae and Thryonomyidae on the one hand and certain South American genera on the other

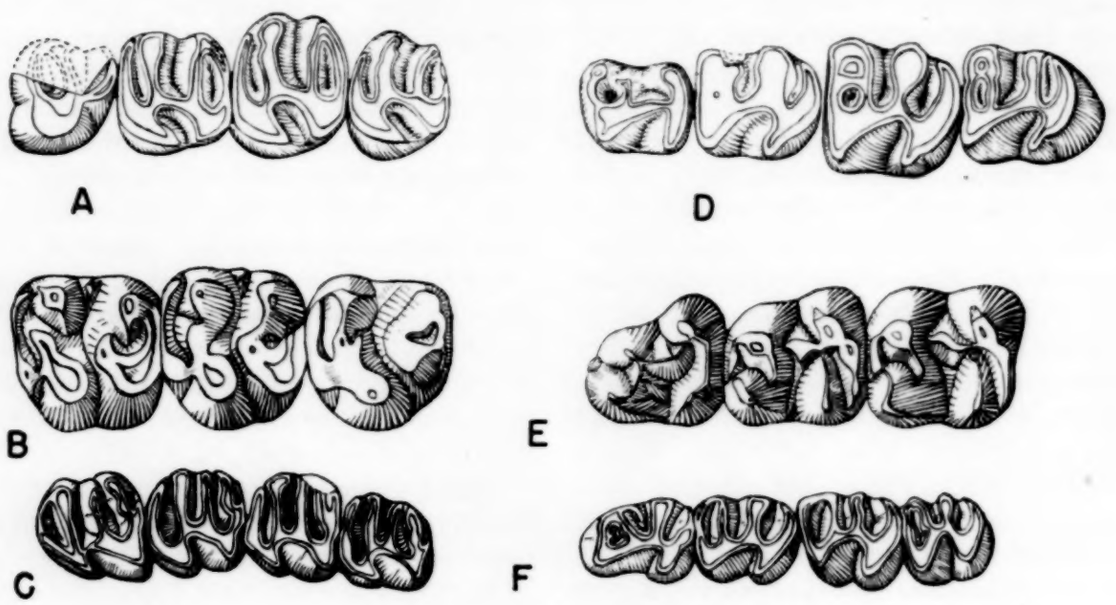

FIG. 1. Teeth of various rodents.

A. *Platypittamys brachyodon.* P^4–M^3 left. Amer. Mus. Nat. Hist. No. 29601, × 10.
B. *Sciuravus nitidus.* M^1–M^3 left. Yale Peabody Mus. No. 13333, × 7.5.
C. *Theridomys aquatilis.* P^4–M^3 left. Brit. Mus. No. 27756*b*, × 5.
D. *Platypittamys brachyodon.* P_4–M_3 left. Amer. Mus. Nat. Hist. No. 29600, × 10.
E. *Sciuravus undans.* P_4–M_2 right. Yale Peabody Mus. No. 13349, × 7.5.
F. *Theridomys aquatilis.* P_4–M_3 left. Brit. Mus. No. 27756*a*, × 5.

A and D from lower Oligocene of Patagonia; B and E from Middle Eocene of Wyoming; C and F from Upper Eocene of France.

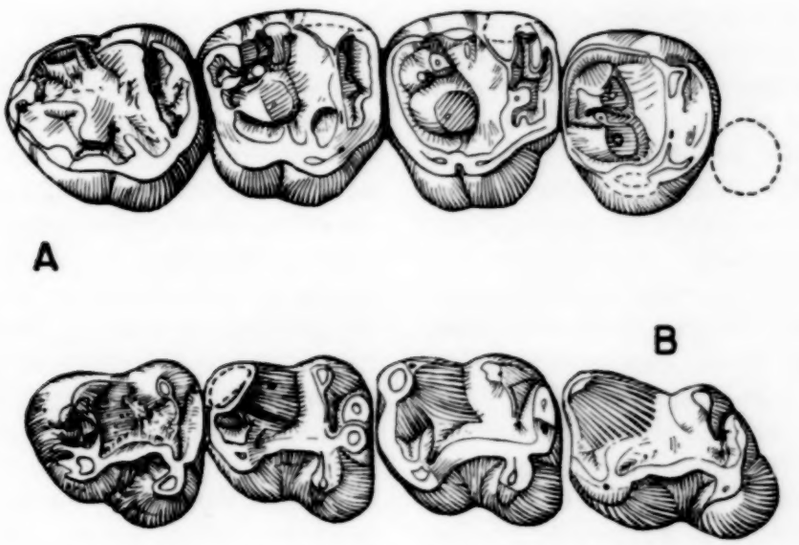

FIG. 2. *Rapamys fricki*, × 5. Upper Eocene of California.
A. dp^4–M^3 right (anterior end to the right). Cal. Inst. Tech. No. 2183.
B. P_4–M_3 left. Cal. Inst. Tech. No. 2181.

are so close that the African families are generally included in the South American Superfamily Octodontoidea (Simpson, 1945, p. 212). For many years they were even placed in South American families, the Octodontidae and Capromyidae. It is worth stressing that the resemblances are not to the South American hystricomorphs in general, but to these two specific families. As mentioned above, the South American families all seem to have been derived from a common stock. The Acaremyidae, represented by the Deseadan *Platypittamys*, seem to be the only slightly modified descendants of such a stock (Wood, 1949, p. 5). The Octodontidae and Capromyidae differentiated from this stock in South America. Therefore, if the resemblances of the African genera are indicative of close relationships, they must be descended from the South American forms, and not vice versa. Moreover, the origin of the African forms must be later than the origin of their two sets of South American ancestors.

Such a derivation could be explained by the exceedingly improbable reverse raft voyage, at a date since the beginning of the Oligocene, of two sets of South American rodents. The apparent resemblances, however, are so striking that Joleaud (1919) used a land bridge with peculiar relationships (Simpson, 1943a, p. 25) to bring the African forms across from South America. For the reasons indicated above, a land bridge seems completely out of the question. Moreover, the unworn teeth of the African *Thryonomys* figured by Friant (1945, p. 201, fig. 1) suggest that the Thryonomyidae at least may so differ from the Capromyidae in the basic elements of the tooth pattern, that it would be difficult to determine the homologies of the parts of the teeth in the African form. It would seem that the apparently identical tooth pattern may be limited to worn teeth. Where this is the case in other rodents, it means that the two groups have been independently derived.

As we know nothing of the past history of either the Petromyidae or the Thyronomyidae, it is equally possible to postulate that they were derived from South American ancestry or that they have developed independently in Africa from an Old World ancestry, and that their resemblances to members of the Octodontoidea are entirely due to parallelism. This may seem to some to be assuming so much parallelism as to be exceedingly

improbable. To the present author, however, the probability of this parallelism appears to be greater than that two sets of rodents were able to float across the South Atlantic. Parallelism would at least seem to be justified as a working hypothesis to direct future morphologic and paleontologic investigations.

Whatever is done with the Thryonomyidae and Petromyidae, it seems almost certain that the South American hystricomorphs and the Hystricidae have been independently derived from the Paramyidae, and that their similarities are due to parallelism and convergence in their subsequent evolution. Presumably, they were not even derived from the same members of the Paramyidae. The hystricomorphs therefore of necessity show considerable parallelism, which increases the likelihood that some of the other troublesome cases may be due to similar parallelism.

Origin of Hystricomorph Characteristics

If the above hypothesis of the lack of relationship between Old and New World hystricomorphs is correct, we should find suggestions of the initial stages of the independent development of subordinal features in the fossil record. An analysis of the available data not only suggests that there were cases among rodents of parallelism of this order, but may even be considered to indicate the probability that this particular parallel development did occur in the case of the hystricomorphs.

Initially, the rodents became separated from other mammals by the development of elongated, chisel-shaped incisors, which were used in gnawing. Gnawing requires a different usage of jaw muscles from other types of chewing, and a number of adaptive modifications of the muscles, skull, and jaws took place. In the Eocene, these had already occurred, and the rodents were a successful group, of Holarctic and perhaps African distribution. The main jaw muscle used at this time was the temporal, which would close the jaw or pull it backward. The largest jaw muscle among recent rodents is the masseter, which pulls the jaw forward as well as upward, thus providing the main basis for gnawing. In the Eocene, the masseter was relatively small. As a result, gnawing efficiency must have been limited, and perhaps the rodents were restricted to the gnawing of relatively soft foods.

During the Eocene the rodents passed through an adaptive radiation, resulting in the development of considerable numbers both of individuals and of species. Toward the close of the period they apparently reached an evolutionary plateau. Almost without exception, the Eocene rodents show the primitive arrangement of the jaw muscles, which must have somewhat limited their dietary potentialities. As a result, although successful within their limitations, the rodents must have occupied rather limited varieties of ecologic niches. Due to the resulting severe competition, there would have developed a strong selection for mutations causing an increase in the size of the masseter muscle, or for a gene causing increased frequency of such mutants (Ives, 1949). Such an increase in size of the masseter would have permitted the gnawing of a wide variety of harder foods and the invasion of a considerable number of new ecologic niches. It seems quite clear that there was what appears to have been a rather sudden development, near the close of the Eocene, in a large number of independent lines of rodents, of modifications of the skull and jaws correlated with an increase in the size of the masseter. This appears to have occurred throughout the northern hemisphere. The apparent suddenness is probably an example of tachytelic evolution (Simpson, 1944, p. 134), although it may in part be due to the fact that we know relatively little of late Eocene rodents.

Primitively, the masseter is attached to the slender zygoma, where there is not enough room for the origin of a great muscle mass. Therefore, if the muscle

enlarged, it must either have increased the thickness of the zygoma, which it did as a subsidiary change in some forms; have migrated up in front of the zygoma onto the side of the snout, which it did in the Sciuromorpha (squirrels, beavers, etc.); have migrated up inside the zygoma, through the enlarged infraorbital foramen, and out onto the face, as in the Hystricomorpha; or a combination of these methods, as in the Myomorpha (rats and mice and numerous other forms).

The greatest single morphological characteristic shared by all the hystricomorphs is this migration of the origin of part of the masseter through the infraorbital foramen onto the face. The structural modifications appear identical or nearly identical in both the New and Old World hystricomorphs. These changes in the masseter are associated with a number of other modifications of the skull, particularly the tremendous enlargement of the infraorbital foramen. The angle of the jaw is also inflected in all forms, which is also related to the activity of the jaw muscles. The teeth of modern hystricomorphs show superficial similarities, but they appear to have been developed by parallel or convergent evolution from different starting points, and the similarities are much less apparent in unworn teeth than in worn ones (Friant, 1935). Other common characteristics, such as the presence of quills, are found in rodents belonging to other suborders.

It is clear that the three main types of modification of the masseter were developed independently though at about the same time. It is also becoming progressively clearer that, within at least some of the major categories, there were independent developments of this type of modification. The squirrel type, with the masseter in front of the zygoma, has been shown to have developed twice independently (Wood, 1937a, p. 195), and may well have been developed four or more times.[6] It seems almost certain that the hystricomorph type developed independently at least in the theridomyids and the New World hystricomorphs, and probin the Hystricidae. In the absence of fossils, it does not seem unreasonable to believe that the enlargement of the masseter in the Petromyidae and Thryonomyidae occurred independently of the changes in the New World, and that the other changes in the skull and jaw were associated with it, and with the corresponding modifications of the rest of the gnawing mechanism.

Parallelism among Porcupines

It has been shown (Wood, 1937b; 1947, p. 158–159) that the rodents exhibit great quantities of parallelism in their tooth evolution. The similarities in tooth structure between the Old and New World hystricomorphs are no more than are known to have occurred elsewhere in the order, among non-related groups. In fact, when unworn teeth are examined, the most striking similarities disappear. Similarities between the Old and New World hystricomorphs go farther than this, however, extending to much of the skeleton as well as the soft anatomy. If the Old and New World groups are not related, it must be a very striking case of parallelism.

According to the present classification, the Old World and New World groups make up the suborder Hystricomorpha, derived from a different and more primitive suborder, and sharing a number of rather complex specializations. While admitting that geographically this is not reasonable, up to the present time no one has been able to discover any very good structural basis on which these two groups of hystricomorphs could be separated on a subordinal level. Nevertheless, as pointed out above, the two groups seem to have been independently evolved.

[6] As this paper is being completed, Wilson's Early Tertiary Rodents of North America has been received, in which he suggests that the use of the structures of the zygoma and masseter for rodent classification may well give incorrect results, due to the same structural modification having arisen several times independently (Wilson, 1949, p. 150, 154–155).

This raises a very intriguing problem, not only for the taxonomist, but also for the student of evolution. Most authors would agree that, if there are two groups which cannot be separated other than geographically, there is no valid basis for separating them taxonomically, although, as pointed out by Simpson (1945, p. 7), geographic distribution is unquestionably a feature of importance to the taxonomist. Likewise, most students would agree that, where two groups can be shown to have been derived from different ancestors, they normally should be separated, taxonomically.

Perhaps this point should be elaborated, as cases can be cited where these criteria do not necessarily hold. Many authors are coming to accept the idea that the mammals have been derived several times from the reptiles, and yet all leave the mammals as a single class (Olsen, 1944, p. 124; Simpson, 1945, p. 17). This is partly because the most advanced of the mammal-like reptiles are called reptiles rather than mammals on rather formalistic grounds. By a slight shifting of the criteria for separating fossil mammals from reptiles, the line could be drawn at an earlier point in geologic time, at which stage all the mammals might have had a common derivation from reptiles. In addition, because there are no other groups of mammal-like animals derived from other groups of reptiles, the chance of confusion is not very great. In those classifications of mammals which include the Miacidae among the Creodontia, there is a probability of polyphyletic origin for the Fissipedia from the creodonts. But again, there are no other groups of carnivores, closely paralleling the fissipedes, descended from other groups of creodonts.

But among the rodents, there are many groups descended, presumably, from the Paramyidae, either directly or indirectly.[7] Temporally, they have all been separated from the ancestral stock almost equal lengths of time. Morphologically, some have become more distinct from the Eocene ancestors than others, and some have evolved in directions all their own, while others have moved in more popular directions, paralleled by a number of other groups. We still do not know from which of the Eocene rodents most of the later families have been derived. As a result, there is no paleontologic basis for believing that the Old and New World hystricomorphs are genealogically any closer to each other than either is, for example, to the Sciuridae or Cricetidae, and considerable basis, on paleogeographic grounds, for believing that they are not.

We cannot solve the problem of rodent classification by doing as we do with mammals as a whole or with the carnivores, and lumping all the specialized descendants of the generalized ancestors in a single group, since such a lumping would bring together all but a single genus (*Aplodontia*) of modern rodents in a single taxonomic unit.

Considering the "hystricomorphs" alone, there are two alternatives. We may separate the New World porcupines from the Old World ones, as a distinct suborder, admitting that there are at present no adequate morphologic criteria for making this separation, and that it would have to be done on a geographic and paleogeographic basis with the hope that additional paleontologic data would demonstrate the validity of such a separation. Or we may leave the Hystricomorpha as a single suborder on morphologic grounds, with the understanding that very possibly the Old and New World forms are more closely related to members of other suborders than they are to each other. Until the problem of rodent classification can be considered as a whole, the latter is felt to be the more conservative course, although probably the more misleading.

[7] The Paramyidae, as represented by their Middle Eocene and later members, are probably too specialized to be ancestral to most rodents. The Lower Eocene paramyids, however, include some forms which seem to be very close to being the common ancestors of all later rodents. Unfortunately, these Lower Eocene forms have not yet been adequately studied.

If these conclusions are correct, this indicates a very interesting problem in the field that might be called paleogenetics. Presumably the Paramyidae, being a single family and at that time of relatively recent origin, had a rather uniform genetic make-up during the Lower and Middle Eocene. Given a world-wide (or at least Holarctic) situation where there was identical or nearly identical selective action of the environment for enlargements of the masseter muscle, and given the very few possible ways in which it could enlarge, it would seem that there followed a series of identical or nearly identical mutations in the two parts of the world. The similarity of these mutations was so great that there resulted a morphologic similarity so close to identity that, to date, it has in some cases successfully defied analysis. Since it does not seem at all reasonable to assume that the structural modifications involved resulted from one or two mutations, the resulting parallelism must have been very great. A similar explanation has been suggested for the distribution of the African and South American frogs of the family Pipidae (Schaeffer, 1949, p. 61).

Perhaps this looks a little like orthogenesis, but it is not intended as such. Gnawing was a very successful acquisition for the rodents, and permitted them to become very numerous and, within their limitations, divergent. However, it also strictly limited the directions of their possible future evolution. If, as has been suggested, there was a subsequent adaptive value in increasing the size of the masseter muscle in order to use these chisel teeth to full advantage, it is not surprising that mutations bringing about this change should have had a positive selective value.

It is possible that only a small number of mutations (perhaps governing the growth rate of the masseter) were involved, or that genes increasing the mutation rate of the masseter genes were present (Ives, 1949). If either of these were true, the likelihood that masseteric mutations would have occurred independently on several occasions is increased. Once such a change had occurred, the associated skeletal changes would have proceeded rapidly.

Since there were a very limited number of ways in which the modifications of the masseter could have been brought about, there would probably have been a strong selective influence acting on any of a small number of possible different mutational trends. That is, we would have orthoselection rather than orthogenesis, and would, as a result, wind up with very distantly related animals which could be distinguished from each other morphologically with difficulty if at all, although they would all be quite different from the ancestral stages.

Summary and Conclusions

The hystricomorphs do not lend support to the hypothesis of trans-Atlantic land connections during the Tertiary. On the contrary, the South American forms can be shown to have definite affinities with the North American Eocene Paramyidae, suggesting an orthodox geographical origin for these rodents. The Old World forms cannot have been derived from the same North American forms, but presumably are descended from Old World representatives of the basal rodent stock.

If this is the case, the similarities between the Old World and New World hystricomorphs must be parallelisms, resulting from similar evolutionary trends in different parts of the world. These similarities are largely in the structure of the jaw muscles and associated regions of the skull and jaws. The similarities in tooth structure are superficial, appearing to be most striking in worn teeth. The Petromyidae and Thryonomyidae of Africa show many resemblances to South American forms. If these are not the result of parallelism, it means that two sets of South American rodents must have floated to Africa.

The variations in the skulls and jaw

muscles, which are now used to separate the various suborders of rodents, do not appear to be primary variants, but each one may have developed independently several times, and thus represent parallelism or convergence.

If the similarities between the Old and New World hystricomorphs are entirely parallelisms, it leaves the taxonomic problem of what to call these groups and how to rearrange the classification of the order. This problem cannot be settled at the present time, as it should be taken up in connection with a re-study of the classificaiton of the entire order.

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NOTES AND COMMENT

A FUNDAMENTAL SEX DIFFERENCE BETWEEN LAGOMORPHS AND OTHER PLACENTAL MAMMALS

GEORGE A. PETRIDES

Characteristics distinguishing rabbits, hares, and conies from rodents have been discussed by numerous investigators, especially Lyon (Smith. Misc. Coll., **45**: 321–447, 1904), Gregory (Bull. Amer. Mus. Nat. Hist., **27**: 1–524, 1910), Gidley (Science, n.s. xxxvi, No. 922: 285–286), Wood (Trans. Amer. Phil. Soc., n.s.; 271–362, 1940), Simpson (Bull. Amer. Mus. Nat. Hist., **85**: 1–350, 1945), and Moody, Cochran & Drugg (Evol., **3**: 25–33, 1949).

An anatomical characteristic of rabbits and hares which seems to have passed unnoticed concerns the position of the scrotum relative to the penis in males. In lagomorphs, the scrotum is anterior to the penis; in rodents and other placental mammals possessing scrota it is usually posterior.

The lagomorph scrotal position resembles that of marsupials. In fact, the anterior scrotal position frequently is given (Gill, Smith. Misc. Coll., **11**: 1–98, 1872) as an ordinal character of the latter group. Though Gill stated that the scrotum is "never in front of the penis" in the placental mammals, Ingles (Journ. Mammalogy, **22**: 227–250, 1941) sketched (p. 241) the true relative positions of the male sex organs, in discussing sex determination in *Sylvilagus auduboni*. Severaid (Bull. Maine Dept. Inland Fish & Game, 1–95, 1942) also prepared a drawing showing the location of the penis in the snowshoe hare (*Lepus americanus*) which precludes the possibility of a posterior position for the scrotum in that species. Neither of these authors, however, seemed to notice the unusual arrangement of the scrotum and penis in these leporids. No published information is available in this regard for species of the Ochotonidae, but I am informed by Dr. H. H. T. Jackson that this anterior position of the scrotum also holds for *Ochotona princeps*.

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EVOLUTION IN THE SOVIET UNION

G. L. STEBBINS, JR.

Since the summer of 1948, when it reached its climactic phase, the Russian controversy over beliefs in the fields of genetics and evolution has been followed with great interest by scientists and laymen alike. Until recently, however, those not familiar with the Russian language and not in touch with the source material have had to rely on second hand information about this controversy, much of which has been clearly biased in favor of one or the other side. Now, however, Dr. Conway Zirkle, the well known historian of science, has provided us with a carefully chosen and skillfully edited volume, in which, through translations from original articles published in various Russian journals, the Russians are allowed to indict themselves.[1]

The particular interest of this book to evolutionists is twofold. In the first place, it documents the stand of the Russians in favor of the Lamarckian theory of evolution, which elsewhere in the world has been completely abandoned as unsupported by factual evidence. Secondly, it shows how, in a totalitarian state, politicians and other men with influence but with little scientific knowledge, can establish by decree and by propaganda the beliefs which all scientists must hold, regardless of any evidence which they may discover in contradiction to them.

In regard to Lamarck's ideas, Dr. Zirkle has recorded a remarkable reversal of opinion during the last ten years. Between 1936 and 1939, the supporters of T. D. Lysenko, the "geneticist" who has led the opposition to Mendel's laws and to other principles of modern genetics, vigorously denied that Lysenko's views have anything in common with those of Lamarck, even though the fundamental basis of both Lysenko's and Lamarck's concepts is the inheritance of characteristics acquired during the lifetime of the individual, through the direct influence of the external environment. Thus M. B. Mitin, head of the Philosophical Institute of the Academy of Sciences, wrote an

[1] Zirkle, Conway, Editor. Death of a science in Russia. Philadelphia, University of Pennsylvania Press. $3.75. See also: Huxley, Julian. Heredity East and West. Lysenko and World Science. Henry Schuman, New York. $3.00.

article in *Pravda* for December 7, 1939, in which his praise for Lysenko included a rebuke to the "formal geneticists" who accused Lysenko of Lamarckianism, and by his language made clear the fact that in 1939 "Lamarckianism" was a bad word.

In 1948, however, not even the Russians could deny the great similarity between the views of Lysenko and those of Lamarck. Therefore, this same M. B. Mitin, speaking before the Lenin Academy of Agricultural Sciences on August 6, 1948, told his audience that Lamarck's teaching, since it arose in connection with the French Revolution, "reflected the revolutionary epoch of the time." Such statements were sufficient to remove the opprobrium previously attached to the term Lamarckianism, and to elevate it to a position of high respectability in Russian thought. To be sure, an earlier speech at the same meeting had brought forward "evidence" in favor of the Lamarckian theory. V. A. Shaumyan, a leading cattle breeder, explained the evolution of increased size in the udder of modern domestic cows as follows. Each cow, during her lifetime, receives 6–7 million strokes from a milkmaid's hands. This cannot fail to stimulate increased growth of the udder, and no one can deny that this stimulation, applied generation after generation, must produce a hereditary increase in the size of the udder!

To those familiar with the brilliant achievements of such Russian scientists as Vavilov, Levitzky, Karpechenko, Chetverikov, Dubinin, and Schmalhausen, all of whom have been killed, exiled, or forced to "recant," the ridiculous mouthings of men like Shaumyan are more tragic than comic.

The influence of politics and "philosophy" on scientific thinking forms the main theme of Dr. Zirkle's documentary. In his introduction, Dr. Zirkle carefully and accurately explains the obscurantism, the raising of false issues, and the verbal tricks by which the cleverer supporters of Lysenko, as well as the politicians who are behind him, confuse the thinking of their audiences and manage to elevate the naive, almost unintelligible utterances of an untrained peasant like Lysenko so that they are regarded as profound scientific truths. Of equal value are Dr. Zirkle's carefully prepared footnotes to the more important translations. These point out specifically the half-truths, the subtle innuendos, and the clever bits of misrepresentation by which the more able speakers achieve their goal. After reading these speeches, one gains the impression that scientific conferences in Russia at the present time resemble the political debates of Tammany Hall in the 1890's, or the efforts of Dr. Goebbels in the 1930's.

After the polemics have all been recorded, Dr. Zirkle prints a translation of an editorial in *Pravda* for August 27, 1948, which to the present reviewer is the most significant document of all in revealing the true aims of the Soviet authorities. In this editorial, objectivity in scientific work is declared bad, and scientists are told that they must regard all of their work from the viewpoint of partisanship. The official decree is that from now on science in the Soviet Union and its satellite nations will no longer consist of an objective, impartial search for the truth. Soviet science has only two objectives. The first is to produce technological improvements for increasing the well being of the Soviet citizen; the second is to obtain additional propaganda in support of the Russian brand of Marxism. In his introduction, Dr. Zirkle suggests the following explanation of why genetics has been eradicated from the Soviet Union: "It is possible that authoritarianism simply cannot allow the existence of the intellectual standards of free scientific inquiry." In this reviewer's opinion, the *Pravda* editorial just cited shows that this explanation is by far the most probable one. In view of this fact, the title of Dr. Zirkle's book might well be shortened to read: "Death of Science in Russia."

INTROGRESSIVE HYBRIDIZATION [1]

CARL L. HUBBS

Given an opportunity in the Wiley Biological Research Series to synthesize methods, facts and ideas, in a field that he himself has especially cultivated, Edgar Anderson surveys in this stimulating little volume the problem of interspecific hybridization in nature, with particular reference to the introgression of genetic traits from one species into another. Objectively and conservatively, yet with enthusiasm and inspiration, he portrays the genetic interplay between species, and shows, significantly, how this interplay is controlled by the environment. In so doing he makes a notable contribution to the facts and interpretations of speciation, though he wisely refrains from sweeping claims or conclusions as to the importance of hybridization as a factor in evolution. In this

[1] Introgressive Hybridization. By Edgar Anderson. John Wiley & Sons, New York, Chapman and Hall, London, 1949: i–ix, 1–109, figs. 1–23. $3.00.

early stage of developing the subject, he is more concerned with measurement and with analysis than with argument and opinion.

On the basis of impressive evidence, Anderson concludes that the end result of natural hybridization is often, if not usually, the modification and increased variability of the parental species, because, if it leads anywhere, such crossing ordinarily promotes backcrossing with one or both species. Thus, gradually, genes may infiltrate from one species into another. Because of the close adjustment of each species to its particular environment and its segregation therein, hybridization occurs chiefly where the environment has been disturbed and hybrid swarms are produced only where man or nature has greatly altered the habitats. The intermediate hybrids will not ordinarily locate a niche and will not survive as such by interbreeding. Those that resemble one or the other species most closely, physiologically as well as morphologically, will tend to occupy and persist in the habitat of that species. There backcrossing will take place and will often continue until the population will be modified and rendered more variable, without losing its specific identity. The gene interplay is roughly proportional to systematic relationship, but may involve genera.

Considerable and welcome attention is given to the bearing on interspecific hybridization of the genetics of multiple factor characters, a subject which Anderson has long pursued and which led him into this idea of introgressive hybridization. The high number of multiple factors that seem to operate in the development of most characters of systematic value is said to yield an F_2 generation much like the F_1, with only moderately increased variability, and is held to render virtually complete the reconstitution of the recurrent species by only five or six successive backcrossings in one direction. The strong linkage of multiple factor characters is claimed to increase vastly the probability of obtaining offspring like the recurrent species. It is suggested that linkage may provide the initial isolation necessary for the action of natural selection.

The conclusions stated by Anderson, on the basis of his studies of hybridizing plant species, ring true and clear to me, not only because of the plausibility of the evidence presented, but also because they apply so very well to the results of my own studies on hybridization between the lower systematic categories of fishes.

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SOCIETY FOR THE STUDY OF EVOLUTION

Report of the Secretary for 1949

Minutes of the Council Meeting at New York, December 27, 1949

The Council, with seven members present, enacted the following items of interest to the Society as a whole:

1) The Editor was authorized to print an edition of Vol. IV of Evolution of 1500, as for previous volumes. Inventory on hand is 380 sets of Vol. I, 467 sets of Vol. II, and 434 sets of Vol. III.

2) In view of the limited supplies of stocks, the Treasurer was requested to sell individual numbers of Evolution only to replace lost copies, in order not to break more sets than necessary.

3) As Evolution is an international journal, the Editor was instructed to submit to the Council any acceptable paper in a foreign language (French, Spanish, Italian, German) for authorization of the extra costs of composition.

4) The Council voted a budget of $5,000 for Vol. IV.

5) The Editor was authorized to draw up a new schedule of reprint costs to authors that would be commensurate with the cost of reprints to the Society.

6) The panel of Associate Editors for the class of 1952 was approved by the Council (published on the cover of this number), and the Editor was authorized to fill any vacancies that might occur between annual meetings of the Council.

With respect to the business of the office of Secretary, the Council made the following actions:

1) It was voted to publish in Evolution an abstract of the financial statement of the Society and abbreviated notes on the essential business of the Society.

2) It was voted to publish a Directory of Members no more often than every two years.

3) The Secretary was directed not to publish abstracts of papers in the program of the annual meeting because of the cost of this practice.

4) The Council voted to meet with the biological societies in 1950. This action probably means that the annual meeting will be with the AIBS at Ohio State University, 11–13 Sept., rather than with the AAAS at Cleveland in December.

5) The President was instructed to investi-

gate the possibility of the Society meeting in California in 1951.

6) An amendment to Art. V of the Constitution, proposed by the Chairman of the 1949 Nominating Committee, was approved by the Council for a mail ballot by the membership.

This amendment called for the addition of the office of President to those of Secretary, Treasurer, and Editor for which single nominations are made. It was defeated by 128 to 73 by a count of votes on March 8, 1950.

The Annual Business Meeting of the Society, December 30, 1949, heard the minutes of the Council meeting and discussed various aspects of the report. The Secretary announced the results of election of officers for 1950. This is printed on the cover of the number of EVOLUTION.

Respectfully submitted,
STANLEY A. CAIN, *Secretary*

SOCIETY FOR THE STUDY OF EVOLUTION

REPORT OF THE TREASURER FOR 1949

(Nov. 6, 1948–Dec. 1, 1949)

RECEIPTS

Balance on hand at last Report		$ 3059.15
Total dues from members (including advance payments)		4263.97
Total subscriptions		2128.14
Payment of reprint bills	$ 577.87	
Sale of back numbers	1059.60	
American Phil. Soc. Grants	2228.64	
Miscellaneous	7.60	
Miscellaneous receipts	3873.71	3873.71
TOTAL 1949 RECEIPTS: $10,265.82 plus balance		$13,324.97

EXPENDITURES

Secretary's Office expense	$ 330.99	
Treasurer's Office expense	304.48	
Bank charges	59.03	
Reprints and postage, Lancaster Press	648.45	
Miscellaneous expenses	1342.95	1342.95
Printing of EVOLUTION		
Vol. II, No. 4	1198.24	
Vol. III, No. 1	1298.82	
Vol. III, No. 2	1174.81	
Vol. III, No. 3	1030.40	
Vol. III, No. 4	1645.33	
	6347.60	6347.60
Total Expenditures		7690.55
BALANCE (as shown on Bank Ledger sheets)		$ 5634.42

(signed) KARL P. SCHMIDT,
Treasurer

CORRECTION

In Volume 3, Number 4, of EVOLUTION there was an error on page 377. Footnote No. 2 of the article by Theodosius Dobzhansky should have read as follows:

[2] CUENOT, L. 1941. Invention et Finalite en Biologie. Flammarion, Paris. 267 pp.

VANDEL, A. 1949. L'Homme et l'Evolution. Gallimard, Paris. 201 pp.